THE ASSOCIATION BETWEEN BLOOD PRESSURE AND VASCULAR CHARACTERISTICS IN CHILDREN

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ABSTRACT

Hypertension is thought to exist in up to five percent of children. A select number of studies have investigated the role elevated blood pressure plays in pediatric atherosclerotic progression. However these studies contain significant methodological flaws and fail to recognize important confounding factors. Therefore, the influence of elevated blood pressure on arterial health in children remains to be clearly understood. The purpose of this study was to investigate the association between blood pressure (BP) and arterial thickness and stiffness in children. Common carotid artery (CCA) intima-media thickness (IMT) and distensibility (Dist), as well as systemic pulse wave velocity (PWV) were measured in 21 elevated blood pressure (EBP; BP $\geq$ 95th percentile) and 83 normal blood pressure (NBP; BP < 90th percentile) children 11-14 years of age. Both EBP and NBP groups demonstrated BP within the normal clinical range, but EBP showed significantly elevated BP as compared to the NBP group. Independent t-tests failed to show significant differences between the EBP and NBP groups for CCA IMT (0.43 $\pm$ 0.05 mm and 0.42 $\pm$ 0.06 mm, respectively) and Dist (0.0058 $\pm$ 0.0024 mmHg^{-1} and 0.0064 $\pm$ 0.0019 mmHg^{-1} respectively). In contrast, a significantly elevated PWV ($p<0.001$) was found in the EBP group (423 $\pm$ 35 cm/s) compared to the NBP group (389 $\pm$ 24 cm/s). This finding remained constant following an analysis of covariance controlling for the effects of maturation, age, sex and obesity. This study shows for the first time that children with elevated BP do not have significantly altered central arterial structure and function as measured through CCA Dist and IMT, but do possess significantly altered systemic arterial stiffness as measured through PWV. This may be the result of sympathetic predominance and its significant influence on the peripheral vasculature. More studies are needed to clearly illustrate the temporal sequence of pediatric atherosclerotic progression in response to elevated BP.

LIST OF ABBREVIATIONS

ABPM	ambulatory blood pressure monitoring
BMI	body mass index
BP	blood pressure
CCA	common carotid artery
CHD	coronary heart disease
CO	cardiac output
cPP	carotid pulse pressure
CV	cardiovascular
CW	continuous wave
CVD	cardiovascular disease
DBP	diastolic blood pressure
Dist	distensibility
EBP	elevated blood pressure group
HC	hip circumference
HDL	high density lipo-protein
HR	heart rate
HRV	heart rate variability
IMT	intima-media thickness
LDL	low density lipo-protein
MRI	magnetic resonance imaging
MAP	mean arterial pressure
MEBP	moderately elevated blood pressure group
NBP	normal blood pressure group
PHV	peak height velocity
PP	pulse pressure
PW	pulse wave
PWV	pulse wave velocity
SBP	systolic blood pressure
WC	waist circumference
WCH	white coat hypertension

Chapter 1 – Introduction

1.1Preamble

Hypertension is one of the leading risk factors for cardiovascular disease (CVD) in adults (37). Twenty-five to 30% of adults are diagnosed with hypertension (42). As for pediatric hypertension, estimates range from approximately 1-5% of children (1; 85). However, it is thought that pediatric hypertension is largely undiagnosed, hence current estimates are likely under-estimated (27). An additional concern is that children with hypertension are likely to become hypertensive adults (10). Also, similar to the adult population, children with hypertension are more likely to be overweight or obese (99, 100). This is of particular importance considering the progressive increase in childhood obesity over the past two decades (46).

In addition to obesity, current research has shown that children with hypertension are at an elevated risk for several other CVD risk factors including elevated low-density lipoprotein (LDL), diminished high-density lipoprotein (HDL), and insulin resistance (33). As well, hypertension has been linked to physiologic changes that lead to cardiovascular morbidity such as left ventricular hypertrophy (42) and diastolic dysfunction (32). Arterial stiffness and thickness have been shown to be surrogate markers of atherosclerotic burden (12). Additionally, these measures correlate with CVD risk factors and predict CV events in adulthood (28; 55). Hypertension in adults has been shown to correlate with arterial stiffness and thickness through a number of scientific investigations (5; 35; 48; 79). However, the affect of elevated blood pressure (BP) on arterial health in a pediatric population is less understood. In fact, only two studies by Litwin and colleagues (50, 51) suggest that arterial thickness and stiffness change in response to elevated BP in children. However, these studies contained a number of limitations including a limited number of subjects in which both males and females, over a broad age range (6-20 years), spanning many pubertal developmental stages and varying body composition profiles were

considered, thus making it difficult to determine the influence that elevated BP itself has on arterial health in children. Therefore, the objective of this study was to investigate the association between elevated BP and arterial health, as measured by central and systemic arterial stiffness and central arterial thickness in a child population after controlling for body composition, sex, age and maturation. It was hypothesized that a higher arterial stiffness and thickness would exist in children with a higher BP compared to those with a lower BP.

Chapter 2 - Review of Literature

2.1Arterial System

The cardiovascular system is comprised of the heart and blood vessels, which are jointly responsible for circulating blood throughout the body. The arterial system is responsible for carrying blood after ejection from the heart, and transporting it to the capillaries delivering oxygen to the working tissue. Arteries are composed of three major layers: the intima layer or tunica interna, the tunica media, and the tunica externa or adventitia. The intima is the innermost layer that lines the lumen of the artery (34). Several studies have shown the intima to be highly involved in the regulation of arterial diameter and elasticity (16; 65). The thick middle layer of the artery, known as the media layer, is comprised of smooth muscles, extracellular matrix, and elastic fibres. This middle layer responds to incoming signals originating from various sources including the intimal layer, liver and autonomic nervous system, in turn causing changes to arterial tone. The outermost layer is called the tunica externa/adventitia and primarily consists of connective collagen and elastin. The heart ejects blood intermittently at very high pressures, but efficient diffusion at the capillaries requires blood to flow slowly at low pressures. In light of this, the arteries are highly elastic and effective at cushioning blood flow to reduce pressure and provide a constant blood flow throughout the capillaries (69).

2.2 The Role of Blood Pressure

Blood pressure generally refers to the pressure exerted on the arterial walls. Systolic blood pressure (SBP) is the arterial pressure (mmHg) created by the cardiovascular system during ventricular contraction, while diastolic blood pressure (DBP) refers to arterial pressure during ventricular relaxation. Systolic blood pressure and DBP pulsate around a mean arterial pressure (MAP), which is considered the steady component of BP and is a product of cardiac output (CO)

and total peripheral resistance (TPR) (95). Both SBP and DBP have been investigated independently in order to further identify risk factors associated with CVD. Historically, because of a poor understanding of cardiovascular disease progression, elevated DBP (which was thought to be more associated with arterial peripheral resistance and the actual pressure within the vascular network) was implicated as the major predictor of CVD (91). At that time, elevations in SBP were correctly interpreted as the consequence of arterial stiffening but the increasing arterial stiffness was thought to be a natural aging process and not atherosclerotic progression (91). However more recently SBP has been found to be more useful in predicting CVD (91). An additional variable which can be obtained from the measurement of BP is pulse pressure (PP), which is simply DBP subtracted from SBP (95). Pulse pressure has been shown to be a powerful predictor of CVD risk (18; 79; 81) and for categorizing hypertensive patients at highest risk for CVD (57). The following sections will focus on the measurement of BP, as well as a discussion of the association between BP and atherosclerosis.

2.2.1 Measuring Blood Pressure

Blood pressure is most commonly measured using the auscultatory technique. This technique includes occluding the brachial artery using a pressure cuff placed around the upper arm. The cuff is then inflated above SBP (30 mmHg above where the palpated radial pulse disappears) and gradually decreased while using a stethoscope to listen for pre-determined sounds. The cuff pressure at the first Korotkoff sound is used for identifying SBP and is simply the appearance of a clear tapping sound in rhythm with the patient's pulse. Diastolic blood pressure, on the other hand, can be recorded at two points during the deflation of the arm cuff. The first option consists of recording the cuff pressure during deflation when sounds become muffled and softer, while the second and more common technique involves recording the corresponding cuff pressure at the

last audible sound as the cuff is deflating (74). For a given individual with SBP equal to 122 mmHg and DBP 84 mmHg, the BP would be presented as 122/84. The Kortotkoff technique requires a highly trained observer, and because of this it is often recorded in a medical setting (74). In fact, in order to clinically diagnose hypertension, there must be three consistent elevated BP measurements recorded on three separate days (74). For individuals over 18 years of age, hypertension is defined as BP above 140/90 mmHg, while pre-hypertension is classified as having BP between 139/89 and 120/80 mmHg. Conversely, hypertension in children is defined as an average SBP and/or DBP that is $\geq 95^{th}$ percentile according to sex, age and height guidelines developed through the National Health and Nutrition Examination Survey (23), while pre-hypertension in children is defined as an average SBP and/or DBP that is $\geq 90^{th}$ percentile, but $< 95^{th}$ percentile for sex, age and height according to the same NHANES guidelines (23). The data used to develop the NHANES percentiles were compiled from a number of large pediatric clinic databases (n>80,000) where each subject had BP measured by a physician (auscultatory) on one occasion.

White coat hypertension (WCH), which is defined as elevated BP in a clinical setting but normal BP at other times (86), has an estimated prevalence of 10-20%. In light of this, BP measurement techniques that require far less training, such as the oscillometric technique, are becoming more commonly used in scientific investigations. Oscillometry record oscillations in BP from an automated sphygmomanometer cuff estimating SBP and DBP from a derived algorithm (61). Studies have shown that BP values obtained by both auscultatory and oscillometric techniques to be comparable (80).

Another emerging technique for the measurement of BP is 24 hour ambulatory blood pressure measurement (ABPM). This method employs portable oscillometric BP measuring devices that measure BP at pre-determined time intervals over a 24 hour period (70). The advantage of this technique lies in its ability to monitor BP during the sleeping hours, to diagnose

silent hypertension and to further avoid misdiagnosing hypertension caused by white coat syndrome (70). In adults increasing BP, as measured through ABPM, correlates positively with increasing end organ damage (98) and ABPM has been shown to be valuable in the prognosis of future CVD events (53). Likewise, when SBP was measured by ABPM in children it was also shown to correlate positively with end organ damage, even when casual BP did not (9; 86). Although ABPM is becoming more common in pediatric health research, its usefulness is under scrutiny because of the lack of reference standards, as well as validity and reliability estimates (53).

2.2.2 Blood pressure and Atherosclerosis

Elevated BP has been found to be related to increased risk of CVD related morbidity and mortality (83). A relationship between increasing BP and coronary heart disease (CHD) was first illustrated by epidemiological evidence reported though the ground breaking and extremely influential Framingham Study. A major Framingham publication by Kannel and colleagues showed that during a 14 year follow-up increasing casual SBP and DBP were related to increasing risk of CHD (38). Further supporting this causative relationship, Kannel and colleagues (37) showed that after following subjects for 18 years 73% of men and 81% of women who had died from CHD had BP >140/90mmHg. A widely accepted theory as to how hypertension detrimentally affects cardiovascular health is through the process of atherosclerosis (69; 18). Atherosclerosis is a compound Greek word comprised of *atheros,* meaning fat deposition or paste and *sclerosis,* which means hardening. Physiologically, this term refers to the gradual accumulation of fibrous tissue, cholesterol and calcium in the arterial walls, eventually projecting into the lumen in the form of an atheroma (18). Severe atherosclerosis can cause arterial occlusion which reduces blood flow and oxygen delivery to tissues downstream. This is

known as ischemia. Prolonged occlusion of cerebral arteries often leads to a stroke, while occlusion of coronary arteries commonly leads to cardiac myopathy, and in severe cases myocardial infarction (18).

The development of sub-endothelial plaque in the arterial walls is a major component of atherosclerosis (5). It is hypothesized that the elevated cyclical stress on the arteries that normally accompanies hypertension causes an enlargement of naturally occurring gaps in the intima layer (18). This increased space between endothelial cells allows circulating LDL to migrate into the sub-endothelial space where they make contact with the media layer. Below the intima layer, LDL is susceptible to oxidation by free radicals as well as proteoglycans, which are an important component of the extracellular matrix. When oxidized, the modified LDL (mLDL) has an inflammatory response releasing cytokines to recruit macrophage migration to the area of intimal damage. In turn, the macrophages engulf the mLDL. However, without a negative feedback mechanism in place, unregulated mLDL accumulation occurs within the macrophages. These engorged macrophages, known as foam cells for their fluffy appearance, contain large quantities of lipids which cannot escape back into circulation to be metabolized or re-utilized by liver. Eventually, foam cells gather to a point where atherosclerosis can first be clearly viewed in the form of a fatty streak, appearing as a yellow smear on the arterial surface (18). The terminal point of atherosclerotic progression involves plaque formation. Transition from a fatty streak to fibrous plaque involves the migration and proliferation of smooth muscle cells, as well as the accumulation of lipids and extracellular matrix within the arterial wall, often with the endpoint of arterial occlusion. (18).

2.3 Arterial Measures

Traditionally, invasive techniques requiring arterial catheters such as angiography and more recently angioscopy (fibre optic imaging of the inner vessel wall), were employed for investigations of arterial adaptations (56). However, recent non-invasive echo-Doppler ultrasound techniques have emerged as useful estimators of arterial structure and function (56). This has allowed for more widespread arterial investigations which commonly include patients such as children, for whom ethical reasons commonly arise when using catheterization for scientific endeavours. The following sections will focus on the non-invasive methodology of echo-Doppler ultrasound and how it is used to measure arterial structure and function. As well, an overview of CV risk factors found to influence arterial structure and function in both adults and children are highlighted.

2.3.1 Echo-Doppler Ultrasound

Echo-Doppler ultrasound technology allows for the non-invasive visualization of arteries (69). The following section will include a discussion the theoretical principles behind Echo-Doppler ultrasound as well the limitations of this technology in order to better understand the implementation of this equipment into investigations of arterial structure and function.

2.3.1.1 Principles

The term "ultrasound" is used to describe any sound which has a frequency above the human hearing threshold. The principles behind Echo-Doppler ultrasound are based on the following equation:

$$f=c/\lambda$$

According to this formula, f refers to the wave frequency, c is the propagation speed of vibration, and λ is the wave length. The propagation speed, "c", in living tissue is generally $1.5x10^5$ cm/sec, while the wave length "λ" ranges from 0.08 to 0.016 mm when frequencies (f) range from 2 to 10 MHz (69). Attenuation or disruption of the ultrasound vibration intensity can result from tissue or scatterers (69). Different tissues can attenuate the ultrasonic vibration in specific ways. For example, a small particle such as a red blood cell scatters the signal in all directions, whereas a larger interface such as a vessel wall results in a specular reflection of the signal (69). Frequency of vibration, homogeneity of the ultrasonic beam, and the absorptive properties of the medium all influence attenuation (69). Therefore for best clarity, more superficial imaging is done at higher frequencies (5 to 10 MHz), while deeper imaging is done at lower frequencies (2 to 4 MHz) (69).

2.3.1.2 Transducers

The transducer serves to generate and also detect the reflected ultrasonic signal. It is a central component to any ultrasound unit. Ultrasonic frequencies are transmitted poorly through materials of low density. Therefore, when transmitting a signal between biological tissue and a transducer, a water-based medium or coupling gel is applied between the two surfaces. Generally, transducers are comprised of piezoelectric ceramics, which permits them the special ability to transform electrical energy into physical or acoustic energy when transmitting the ultrasonic signal. In turn when receiving the signal, transform that physical acoustic energy back into an electrical signal (69).

The transducer transmits the ultrasound signal along a beam. The dimensions of the beam are a result of the size and shape of the transducer, as well as the frequency of operation.

Additionally, the signal received is limited to the properties of the ultrasound beam. Typically, beam widths range from 2 to 10 mm (69).

Two types of transducers exist. The first and simplest type is what is known as a continuous wave (CW) transducer. Initially used in the mid 1950s, the CW technique uses two separate transducers: one to continually transmit ultrasound vibrations, and the other to continually receive reflected (back-scattered) signals (69). CW ultrasound has more accuracy when estimating high-velocity blood flow, is relatively in-expensive and has simpler electronic circuitry. On the other hand, CW ultrasound lacks depth selectivity which can be disadvantageous when working with a large sample volume, and difficult to obtain a desired signal when vessels are close together (69). The second type of transducer is pulse-wave (PW). PW ultrasound uses only one transducer that serves to both transmit and receive the ultrasonic vibrations. As the name suggests, PW uses interrupted pulses of ultrasound (pulse repetition frequency) containing 3- 20 cycles at a given frequency (69). In order to determine if a signal originated from a fixed depth, the PW ultrasound transducer uses a range-gate delay. The number of cycles transmitted and time the range-gate is open determines the sample size. As well, beam width of the probe can help to determine sampling volume (69). All these characteristics of PW ultrasound allow for the localization of a well-defined source, preventing interference from adjacent vessels or structures. Both CW and PW Doppler ultrasound create essentially the same signal. However, since PW has the ability to be very selective in what it images, it is the ultrasound of choice for arterial imaging (69).

2.3.1.3 Limitations

There are several limitations in using Echo-Doppler ultrasound for the purpose of obtaining arterial images. Most problems result from technical error, therefore having a skilled operator is

very important. First, keeping the probe parallel with the artery allows for consistent, accurate analysis of arterial diameter changes across the full cardiac cycle. Second, the operator needs to be careful to maintain a minimum hold-down pressure during image collection as this can influence the pulsatile changes in vessel diameter over the cardiac cycle and therefore impede accurate measurement. Third, electrical noise can be caused from within the Doppler system itself, or from external sources. Using high-end equipment can mitigate electrical noise, but there is always a baseline level of noise due to thermal effects (69). In fact, an accurate Doppler signal relies on a good signal-to-noise ratio, making the choice of probe frequency important; for example, high frequencies suffer from severe absorption when being used to image deep vessels, increasing the signal-to-noise ratio (69). Understandably, obtaining a clear, crisp image is paramount during Echo-Doppler collection for accurate analysis. Attempting to minimize the above limitations is essential in order to obtain high quality images.

2.3.2 Arterial Structure and Function

Through modern technological advances, Echo-Doppler ultrasound is now a common tool for measuring arterial properties such as stiffness and wall thickness. A reduction in arterial stiffness is one of the earliest cardiovascular changes to occur in the sequele of atherosclerosis and has been shown to be associated with elevated BP, obesity, insulin resistance, and cardiovascular morbidity (55). Two major indices of arterial stiffness are arterial *distensibility* and *pulse wave velocity* (PWV). As mentioned above, a major function of the arterial system is to cushion and smooth the turbulent flow of blood ejected from the heart (69). Arterial stiffening not only reduces the buffering and smoothing capacity of the arterial wall but often leads to miniscule discontinuities in the artery luminal lining allowing LDL migration into the arterial wall initiating

atherosclerotic plaque formation (18). For these reasons, both distensibility and PWV have been shown to correlate with advancing atherosclerosis and predict future CVD events (5; 6; 11; 81).

2.3.2.1 Arterial Distensibility and Pulse Wave Velocity

Arterial distensibility is given by the formula:

$$Dist = [(sCSA - dCSA)/dCSA] / (Ps - Pd)$$

Where Dist represents distensibility ($mmHg^{-1}$), sCSA and dSCA equals systolic and diastolic arterial cross-sectional area ($cm^{2)}$ respectively, and Ps – Pd represents the pulse pressure (mmHg) within the same or comparable vessel. Distensibility is defined as a relative change in vessel diameter/area for a given change in pressure. For example, an elastic and highly distensible balloon would expand larger and easier as compared to a very stiff balloon when inflated at matching pressure. It is important to note that arterial distensibility can vary within the same artery, and between different arteries depending on proximity to vessel branches, and other factors (69).

PWV velocity is given by the formula:

$$V = D/T$$

Where V denotes velocity (cm/s), D equals the estimated distance the blood travels between two points (cm), and T equals the duration of time required for the pulse wave to travel between those two points (s). PWV therefore, is the speed of blood propagation. When the heart ejects the contents of the left ventricle into the aorta, a pulse wave is produced that travels throughout the arterial system. A healthy and relaxed arterial system propagates the pulse wave relatively slowly through the body, whereas a stiffer arterial system that is unable to store

pulsatile energy in the distension of the arterial wall can only release the pulse energy in the forward plane, resulting in faster pulse propagation speeds. (72). Commonly, PWV is measured between the carotid and brachial, carotid and femoral, (72) or femoral and dorsal pedis segments. (3). In general, PWV estimates *regional* arterial stiffness, whereas Dist describes arterial elasticity in a *localized* arterial section.

2.3.2.2 Arterial Wall Thickness

The process of atherosclerosis not only makes the walls of the arteries stiffer, it also makes them thicker (71). As shown in Figure 1, a common technique to estimate arterial wall thickness is to inspect ultrasound images in order to measure the thickness between the luminal surface of the intima and the inner media (IMT) layers (14).

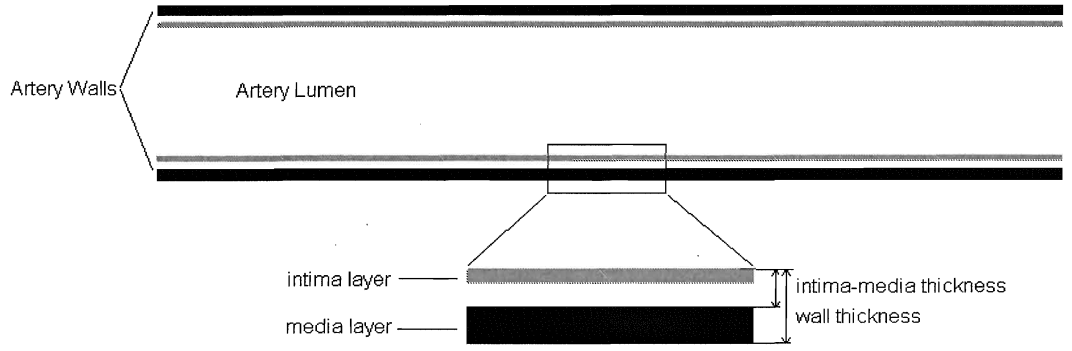

Figure 1. Diagram illustrating major arterial landmarks used for establishing intima-media thickness and

2.3.3 Arterial Measures in Adults

Several studies investigating the adult population have shown arterial properties to be influenced by advancing age (89), obesity (21), sex (49) and BP (41). A study performed by Stensland-Bugge et al. measured IMT in 6408 men and women between the ages of 25-84 years (89) and found it to be positively related to advancing age. The IMT increase with age, however, was greater in men than women. A possible explanation for this lies in an investigation by Mendelsohn and colleagues that showed the feminizing hormone estrogen to improve HDL/LDL ratio, in turn possibly reducing sub-endothelial tissue accumulation (63). In investigating the effect of obesity on arterial stiffness in adults, Danias and colleagues used magnetic resonance imaging (MRI) for measurement of aortic elasticity in male participants aged 20-40 years (21). They found abdominal aortic elasticity to be decreased in their obese population versus normal weight controls (23). Similar findings were found in women by Mizia-Stec et al. who investigated the differences in arterial structure and function between 22-obese and 34-normal weight women (68). PWV and common carotid artery (CCA) β-stiffness index (an additional measure of arterial stiffness) were shown to be elevated in the obese women after matching for SBP and DBP, which can also independently alter arterial measures (68). In fact, a comprehensive investigation performed by Kovaite and others found that after accounting for many of the risk factors for metabolic syndrome in 186 health asymptomatic healthy adults, BP was the most important determinant of arterial structure and function as estimated by IMT and PWV (40). According to this evidence, it is clear that age, obesity, sex and BP influence arterial thickness and stiffness in the adult population.

2.3.4 Arterial Measures in Children

The advent of non-invasive techniques to evaluate arterial health has allowed clinical pediatric investigations to become more commonplace. Several studies in children have investigated the impact that various factors such as age, maturation, sex and obesity have on arterial structure and function (2, 3, 7, 11, 21). However the independent atherosclerotic effect of increased BP is difficult to investigate because of its close association with obesity, advancing age, maturity and sex. A well designed study with relevant and valid measurement of confounding risk factors is needed to clearly demonstrate the independent relationship between BP and atherosclerotic progression.

2.3.4.1 The Effects of Childhood Aging

Autopsy studies have demonstrated that in all children the process of atherosclerosis is initiated in childhood and progresses with age (87). Using non-invasive ultrasound, Tomoko Ishizu et al. measured the IMT of 60 healthy children aged 5-14 years with normal serum lipid concentrations and BP and showed IMT to increase in a linear manner with increasing age. This effect remained after controlling for the influence of sex, obesity, blood lipid profile, parental smoking history and BP, hence these authors suggest that the increase in IMT observed in their study was the result of normal arterial development and not pathologically exacerbated (31). Likewise, Cheung and colleagues investigated arterial stiffness though the measurement of PWV in children aged 6-18 years. They found after controlling for body height, mass and BP, a significant positive relationship between PWV and age existed (19). Another interesting study looking at four age groups from childhood to young adulthood (7-14 years, 11-14 years, 15-18 years and 19-22 years) illustrated that CCA distensibility decreases from childhood to adolescence (47). The above studies together imply that in children, advancing age is indeed related to increased arterial

wall thickness and stiffness. Advancing age therefore, is a factor that must be controlled for when investigating the independent effect of elevated BP on arterial thickness and stiffness.

2.3.4.2 The Effects of Sex

As in adults, arterial tissue in children is speculated to respond to sex hormones. Puberty is a time of rapid hormonal change and peri-pubescent children endure rapid increases in sexualizing hormones (29). The hormones most commonly attributed to sexual maturation in males and females are testosterone and estrogens respectively, and are thought to have differing effects on arterial properties and function (29). Ahimastos et al. published research comparing arterial PWV between four groups; pre-pubescent and post pubescent males and females. Before puberty, females had significantly elevated central and peripheral PWV as compared to males, indicating stiffer arteries. However, post-pubescent females had significantly lower arterial stiffness than their pre-pubescent counterparts, while the opposite was true for males. These changes in PWV resulted in no difference between sexes post-puberty (3). Reduced arterial stiffness in females' post-puberty could be the result of estrogens' ability to reduce smooth muscle proliferation (97), improve extracellular matrix composition (20) and increase vaso-relaxatory nitric oxide (16). In contrast, the increase in arterial stiffness observed in the post-pubescent males could be the result of an increase in testosterone and its' promotion of smooth muscle cell proliferation and monocyte adhesion to endothelial cells (62). Evidently, sex is an influential modulator of arterial properties in childhood and investigations attempting to measure the influence of risk factors such as elevated BP should control for its influence.

2.3.4.3 The Effects of Body Composition

In light of obesity being a known risk factor for CVD in adults and children (44), the relationship between increasing adiposity and arterial properties has been investigated in children (24; 30; 39). Tounian and colleagues were one of the first investigators to directly investigate arterial estimates of atherosclerotic progression in obese children. They found obese children to have significantly reduced arterial elasticity and endothelial function, but no difference in IMT (92). In a similar follow up study looking at only pre-pubescent obese children, these authors once again showed reduced endothelial function as well as arterial elasticity, but no change in IMT (2). Interestingly, a study which measured arterial wall thickness in 96 obese and 25 non-obese children aged 9-13 years showed CCA IMT to be significantly elevated in the obese children (77). This same study however also showed that elevated BP and chronic inflammatory markers played a role in the IMT difference between groups. Lastly, Mimoun et al. showed that even when looking at only an obese population (n=384) of children (age range, 2.5-18 years), the univariate correlation between BMI z-score and arterial distensibility was almost significant at a P value of 0.07 (66). It is clear based on the literature that obesity plays an important role in the thickening and stiffening of arterial tissue in children and is an essential factor that must be measured and controlled for when investigating the atherosclerotic effects of other risk factors such as elevated BP.

2.3.4.4 The Effects of Childhood Blood Pressure

Although the negative impact of CVD risk factors such as advancing age, obesity and elevated BP is well established in adults (18), far less is known about the arterial health of children living with the same risk factors. It has been shown through post mortem autopsies that the majority of children, and in some cases children as young as 8 years old, have atherosclerotic streaks and that they progress in size and frequency with age through adolescence (87). The relative influence of a

number of established CVD risk factors on arterial health, such as elevated BP, have yet to be elucidated in a childhood population. Using non-invasive techniques such as Echo- Doppler ultrasound and PWV, it is now possible to non-invasively study the influence of CVD risk factors on arterial health in children.

In the study by Aggoun and colleagues (2), BP was found to correlate with flow-mediated dilation, a measure of endothelial dependent dilation in pre-pubescent children aged 7-9 years. These authors reported that as BP increased, flow-mediated dilation decreased, suggesting that elevated BP has a negative impact on endothelial function (2). Similarly, Litwin et al. (51) also attempted to investigate the influence BP had on arterial health in children. These researchers showed an increased CCA IMT and decreased distensibility in their hypertensive population (51). In a follow up study, Litwin and colleagues again illustrated a correlation between carotid IMT and BP, specifically SBP and PP (50). Extrapolating results from these investigations is difficult however, as the age ranges used were 6-20 years and 5-18 years respectively. Causing additional scepticism of their results is the alarming admission that the authors were not blinded to the subject's BP status when analyzing arterial images. Echo-Doppler arterial analysis is somewhat subjective due to both the choice each operator has on image and landmark selection using digital callipers (4). This subjective nature combined with the lack of blinding in the aforementioned studies could possibly invalidate their results. (4).

When using a more narrow age range the relationship between childhood BP and arterial health is less clear. Lande et al. investigated arterial parameters of children (age 14.9 ± 2.3 years) and showed a significantly elevated CCA IMT in their hypertensive population when matched with normotensive children based on BMI (43). However, in a similar study using a population on average only one year younger (mean age 13.9 ± 2.9 years), Sorof and colleagues failed to show a relationship between BP and carotid IMT, but did show a significant correlation between BMI and carotid IMT (85). It is also notable that the aforementioned studies (43; 50; 51) not only

didn't control for the effects of maturation and sex, they also used ABMP to measure BP. This technique, although gaining popularity, is rarely used in a clinical setting, so there is limited relevance of these results to physician guidelines for risk stratification according to a child's routine casual BP measurements.

Pulse wave velocity, a more regional estimate of arterial health is widely used in clinical research. A study by Blacher and colleagues revealed that at a given age, PWV is a more powerful predictor of atherosclerosis than left ventricular hypertrophy, hypertension, plasma lipids and glucose, and smoking duration (11). However, these risk factors are known to be clustered in individuals at risk for CVD, suggesting that the PWV predictive power may simply be the accumulation of each independent risk factors affect on arterial health and not an independent risk of its own. Hence, Li et al. looked at how risk factors measured in asymptomatic healthy children related to PWV measured in young adulthood. They concluded that SBP in childhood significantly correlated with PWV measured an average of 25 years later (48). To my knowledge, a study that investigates the simultaneous relationship between PWV and BP in a pediatric population does not exist.

Therefore, it is apparent due to voids in the literature and prior investigations that the relationship between arterial health and BP in a child population is still unknown. This information is important not only in that it may aid clinicians in identifying BP profiles that suggest elevated atherosclerotic risk, but it will also shed light on the progression of *BP associated atherosclerosis* which may allow for a more focused approach towards combating the arterial effects of elevated BP in childhood. The independent effect of BP on arterial distensibility, IMT and PWV in children, while controlling for a number of confounding factors (i.e. age, sex, maturation, BMI) has yet to be investigated.

2.4 Objective

The purpose of this study was to investigate the relationship between BP and vascular characteristics in a pediatric population.

2.5Hypothesis

It is expected that children with elevated BP will have reduced arterial stiffness when compared to their lower BP counterparts. Additionally, it is hypothesized that arterial wall thickness, as estimated by IMT, will be greater in our elevated BP group in comparison to the lower BP controls.

Chapter 3 - Methods

3.1 Study design and sample

The following study was a cross-sectional analysis of children aged 11-14 years carried out in accordance to an agreement with the Niagara Catholic District School Board, and was approved by the research ethics committee of Brock University (Appendix 1). This agreement allowed access to 50 regional schools for the purpose of screening their students for elevated BP. A total of 1913 children had their BP assessed in their school environment using the BPM-300 one step BP monitor (VSM MedTech Ltd. Coquitlam, BC), which has shown to be highly valid and suitable for BP measurements in both adults and children (59). For simplicity, blood pressure was consistently measured on the right arm to avoid the effects of coarctation, as well blood pressure is not systematically influenced by choosing the dominant or non-dominant arm (74). Cuff size was based on arm circumference with at least 80% of the cuffs bladder encircling the arm and a cuff width that was at least 40% of mid-arm circumference (93). The inside of the cuff was marked with an index and range line. If the index line was not within range, or the cuff size was insufficient, the cuff with the appropriate circumference size was chosen. Sizes ranged from child, adult small, adult regular, or adult large. Blood pressure was measured following 15 minutes of relaxed sitting with feet flat on the floor, hand supinated, arm relaxed with the cuff positioned at heart level. Six independent sequential measurements were then taken one minute apart in accordance with the European Youth Heart Study protocol (78). According to work by Graves and colleagues (26), the first measurement was used to familiarize the subjects with cuff pressurization and hence was discarded, while the closest 3 measurements out of the remaining 5 were used to calculate an average BP. Elevated BP (EBP) in children was defined as BP $\geq 95^{th}$, moderately elevated BP (MEBP) was $\geq$90th percentile and $< 95^{th}$ percentile, while normal BP (NBP) was defined as BP<90th percentile for either SBP and/or DBP according to our school sample screening results and adjusted for age, sex and height. Height (cm), sitting height (cm),

waist circumference (WC; cm), hip circumference (HC; cm) and weight (kg) were also measured. From this WC/HC ratio and BMI (kg/m^2) were calculated.

3.2 Laboratory Protocol

From the total sample of 1913 children, 221 students were selected for laboratory analysis. Based on initial field BP measures, there were 71, 47 and 103 students stratified by SBP and/or DBP into each of the EBP, MEBP, and NBP groups, respectively. The EBP and MEBP groups consisted of all participants that were identified as either EBP or MEBP in the school screen that were available for the follow-up laboratory testing, while the NBP group was comprised of randomly selected participants.

Participants were informed of the laboratory testing procedure over the phone before consenting and scheduling a testing appointment. Each lab testing session was approximately 90 minutes and began in the morning with the participants coming into the lab following a 12 hour fast. Upon arrival to the laboratory, subjects were provided a more detailed explanation of the procedures and were required to sign a consent form. Once consent was obtained, each subject provided a pin prick blood drop from the middle finger tip of the non-dominant hand for blood lipid screening. Basic anthropometry was measured before the participant was provided with a standardized breakfast consisting of a juice box, fruit bar and bagel with either jam or cream cheese (~350 kcal). A standard breakfast is important as dietary intake is suspected to have acute affects on arterial properties (73). Also, because a full bladder is known to increase sympathetic activity (22) and hence BP, participants were encouraged to urinate and then rest for 15 minutes in an upright seated position following breakfast. After this point, BP was measured six times using the same protocol as in the schools. Following BP measurement, subjects were required to lie supine for 15-20 minutes while being prepared for the next procedure. Five minutes of supine rest was required in order to record beat-by-beat heart rate (HR), BP and PWV. As well, an

additional five minutes of continuous data collection was required to measure carotid artery ultrasound images, pulse pressure, and left ventricular imaging. It should be noted that arterial analysis was performed as part of a larger study, with several other measures being collected during each testing session. The various data collection sessions were temporally sequenced as not to influence one another.

3.3 Laboratory Procedures

3.3.1 Anthropometry and Pubertal Maturation

Standing height, sitting height, WC, HC and weight were measured identical to the field testing procedures. After removal of footwear, standing height was collected while each subject had their back to a stadiometer (Ellard Instrumentation Ltd. Monroe, WA). Each participant was asked to perform a deep inhalation and exhalation after which the distance from the ground to a point on the wall parallel to the floor on the top of the head was recorded as standing height (0.1 cm). Weight (0.1 kg) was recorded with participants wearing shorts and t-shirts using an electronic calibrated scale (Zenith, Dalbridge, South Africa). Body mass index (BMI) was then calculated using the weight divided by height squared (kg/m^2). Sitting height was collected using the same stadiometer as standing height. Each subject was asked to sit on a table of which the height was known with their back as straight as possible. Sitting height was recorded as the difference between recorded height and table height (0.1 cm). Leg length was calculated as the difference between standing height and sitting height.

Pubertal maturation was estimated from measurements of sitting and standing height entered into a prediction equation developed by Mirwald and colleagues to differentiate pubertal development (67). This variable is referred to as age of peak height velocity (PHV) and represents a developmental landmark where the torso is elongating at the same velocity as the legs (8).

Therefore, "years from PHV" represents the number of years before (represented in a negative number) or after (represented in positive number) PHV occurs, which is roughly at 11 years in females and 13 years in males (36) (See Appendix 5 for formula).

3.3.2 Arterial Distensibility

Five minutes of beat-by-beat pulse wave data taken from the left middle toe (Pulse Oximeter, PB-11341031, Nellcor, Boulder, CO) along with a one-lead electrocardiogram (ECG) waveform was collected. As well, continuous SBP, DBP and MAP were collected through the use of a Finapres (Ohmeda, 2300, Netherlands) positioned at heart level on the left middle finger. Three manual BP measurements were also taken on the right arm at the beginning and end of five minutes of resting supine data collection. Finapres BP values were adjusted to match manual BP recordings to ensure accuracy, after which PP was calculated using both SBP and, DBP (used for the calculation of distensibility). Average SBP, DBP, PP, and MAP were calculated from beat-by-beat data collected during the last minute of the five minute supine rest period. All beat-by-beat data was collected using ADInstruments PowerLab Version 5.1 and ADInstruments Chart 5 Version 5.5.5 (ADIntruments, Colorado Springs, CO).

Following beat-by-beat data collection, resting central arterial distensibility was measured at the CCA. This artery was chosen for its large size, easy access, and relative simplicity in imaging and analysis compared to other arteries. A minimum of three non-invasive imaging sequences consisting of five beat-by-beat diameter changes in the right CCA were recorded using Echo-Doppler ultrasound (Vivid i, General Electric Medical Systems, Netherlands) from a location approximately 2 cm proximal to the carotid bulb. Simultaneously, BP wave contours were collected from the left CCA using a hand-held tonometer (SPT-301, Millar Instruments, Houston, TX). The same investigator (who was blinded to the BP and BP group of all participants) performed all diameter measurements corresponding to systole (peak of

T-wave) and diastole (bottom of S-wave) using the calliper option in the EchoPAC software (ECHOPAC7-002308, General Electric Medical Systems, New York). Three diameters from the two beat images with the highest clarity (as interpreted by the analyst) within each of the five-beat recordings were averaged, resulting in an average of 18 measurements for each of diastole and systole. Pulsatile cross sectional area (CSA; $\pi r2$, where r = radius), and the corresponding pulsatile pressure was used to determine vessel distensibility using the standard equation:

$$\text{Dist } (mmHg^{-1}) = [(sCSA - dCSA)/dCSA] / (Ps - Pd)$$

where sCSA and dCSA represent systolic and diastolic cross-sectional area and Ps and Pd are systolic and diastolic Finapres pressures, respectively. Distensibility was used instead of compliance in order to control for the influence of BP on resting (diastolic) diameter. As well, average finger SBP and DBP were used to calculate distensibility instead of tonometer obtained CCA pressure values. Although left CCA PP was obtained using tonometry, it was not used to calculate distensibility due to the fact that it did not consistently meet the criteria of Chen and colleagues, who reported the need of a stable baseline, clear maximum amplitude and a reasonable configuration in order to accurately estimate intra-arterial pressure changes (17). Although finger BP is known to be augmented compared to more central arterial BP (69), it still provides the most accurate estimate available of the beat-by-beat difference in PP between the groups for this study.

3.3.3 Intima-Media Thickness

The far wall taken from the top of the ultrasound image had more clarity and therefore was used for IMT (mm) measurements. The two highest quality recordings with the three best quality beat images were used for IMT measurement. To ensure the lowest possible tension on the arterial wall, all measurements were completed during diastole. Intima media thickness is the distance

between two specific landmarks within the arterial wall measured perpendicular to the longitudinal axis of the artery. The first landmark is the luminal surface of the intima layer, while the second is the intima-media interface (Figure 1). For each of the two images the common carotid artery IMT was measured at three locations along the length of the artery for each of the three selected beats. Therefore, reported IMT for each subject was an average of 18 separate measures.

3.3.4 Pulse Wave Velocity

In order to estimate PWV over a very large systemic region, it was measured by taking the R-wave of the ECG as the starting point of our pulse wave and using the upstroke of the pulse wave taken from the left middle toe as our terminal endpoint. The R-wave of the ECG was used as the starting point for its accuracy and simplicity of analysis, while the onset of the pulse wave at the toe was used instead of the femoral artery to ensure comfort of our participants. The distance between the sternal notch and middle toe was used as the segment length (cm). The PWV estimate for each subject was obtained by averaging a total of 15 beats.

$$\text{PWV (cm/s)} = \text{segment length/transit time}$$

3.4 Reliability Analyses

In order to test for intra-observer reliability, changes in systolic and diastolic lumen diameters, as well as IMT were assessed on two occasions. Images from 20 participants were randomly chosen 30 days after the initial measurements. Intra-observer reliability of arterial diameter and IMT

showed very high intraclass correlation coefficients for systolic diameter, diastolic diameter and IMT at 0.99, ($p<0.001$), 0.96, ($p<0.001$), and 0.89 ($p<0.01$) respectively (Appendix 7).

3.5 Statistical Analysis

After obtaining a second automated BP measurement in the laboratory, it was decided that only those subjects who consistently remained within the EBP and NBP groups would be analyzed. The reasoning behind this stems from the observation that many participants did not stay within the same percentile range during laboratory BP collection in comparison to the initial school screen, putting the initial categorization of those participants in question. In addition, after removing participants that changed BP categories, only 4 participants remained in the MEBP group, not enough to use for statistical purposes (Figure 2). Therefore, a total of 21 EBP and 85 NBP were analyzed and used for statistical comparison. In the case of PWV, a systematic methodological oversight resulting in sternal notch-toe segments to not be recorded in several participants resulted in PWV being calculated for 71 participants. As for distensibility and IMT, attrition was caused by the inability to collect any quality images on one participant and image quality being greatly distorted during systole for another participant, leaving us with 104 and 105 participants to analyze for each of those arterial properties respectively. As for brachial BPs reported in this study, they were calculated as the average of the field and lab oscillometric BP values.

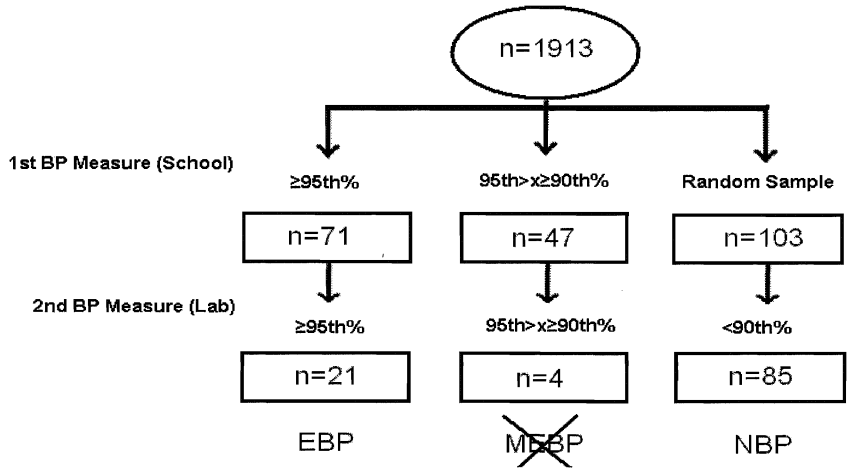

Figure 2. Population sample flow chart for EBP (elevated blood pressure), MEBP (moderately elevated blood pressure and NBP (normal blood pressure)

All statistical analyses were carried out using SPSS software version 16.0 for Windows (SPSS Inc., Chicago, IL). All variables are reported as mean ± standard deviation and significance was set at $p \leq 0.05$. An independent t-test was performed to determine group differences for age, height, weight, BMI, PHV, SBP, DBP and MAP, among the EBP and NBP group. Additional independent t-tests were performed to evaluate differences in arterial characteristics between the EBP and NBP groups. As well, a one-way analysis of covariance (ANCOVA) controlling for sex, PHV, age, and BMI were used to determine group differences for CCA distensibility, IMT, PP and PWV. Also, correlation analysis was performed with CCA distensibility, CCA IMT and PWV as the dependent variables.

Chapter 4 – Results

4.1 Anthropometric and Blood Pressure Characteristics

Anthropometry and BP measurements are shown in Table 1. Significant differences in measures of obesity were found between the two groups ($p<0.001$), as well as average SBP ($p<0.001$), DBP ($p<0.001$) and MAP ($p<0.001$). No significant differences were found between BP groups for PHV ($p=0.89$), age ($p=0.16$) or height ($p=0.26$).

Table 1. Physical and maturational characteristics of normal blood pressure (NBP) and elevated blood pressure (EBP) children

	NBP (n=85)	EBP (n=21)
Demography		
Age (years)	12.8(0.8)	13.0(0.9)
PHV (yrs from PHV)	-1.71(0.81)	-1.68(1.04)
Sex (%male)	34	48
Anthropometry		
Height (cm)	158.0(9.3)	160.6(9.4)
Weight (kg)	46.7(11.6)	65.1(19.6)*
BMI(kg/m^2)	19.8(3.6)	26.4(7.1)*
Blood Pressure		
SBP (mmHg)	91(5)	110(6)*
DBP (mmHg)	55(5)	71(5)*
MAP (mmHg)	67(4)	84(4)*

Mean (SD); *Independent t-test $p<0.001$ significant difference between groups, PHV=Peak Height Velocity, BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, MAP=mean arterial pressure.

4.2 Arterial Characteristics

Characteristics of arterial structure and function are shown in Table 2. Independent samples t-tests showed significant differences in only PWV (cm/sec) ($p<0.001$) and PP (mmHg) ($p<0.01$) between NBP and EBP. No significant difference was found between BP groups for any other arterial measures ($p>0.05$).

Table 2. Arterial characteristics of normal (NBP) and elevated blood pressure (EBP) children

	NBP (n=83)	EBP (n=21)
Arterial Diameters		
SystDiam (mm)	5.43(0.43)	5.6(0.38)
DiastDiam (mm)	4.83(0.43)	4.94(0.39)
DiamDiff (mm)	0.61(0.11)	0.61(0.11)
Arterial Stiffness		
Distensibility ($mmHg^{-1}$)	0.0064(0.0019)	0.0058(0.0024)
PP (mmHg)	44.3(10.8)	51.8(12.5)*
PWV (cm/sec)	389.2(24.0) (n=57)	423.1(35.0) (n=15)**
Arterial Thickness		
IMT (mm)	0.42(0.06) (n=84)	0.43(0.05)

Mean (SD); Independent t-test *p<0.01, **p<0.001 independent t-test, significant difference between groups, SystDiam = arterial diameter during systole of the common carotid artery (CCA), DiastDiam = arterial diameter during diastole of the CCA, DiamDiff=SystDiam – DiastDiam, Dist=Distensibility of the CCA, PP= Finapres obtained pulse pressure, PWV=Pulse wave velocity, IMT=CCA intima-media thickness.

4.3 Determinants of Arterial Properties

Univariate correlations demonstrated that IMT of the common carotid artery was not significantly correlated to any of our measured variables ($p>0.05$), while CCA distensibility was significantly related to PP ($p<0.001$). Further, PWV was significantly related to SBP ($p<0.001$), DBP ($p<0.001$), sex ($p=0.02$), PP ($p=0.01$), BP group ($p<0.001$), BMI ($p<0.001$), and weight ($p<0.001$) (Table 3).

Table 3. Univariate correlation coefficients (r) between independent (x-axis) and dependent variables (y-axis)

	SBP (mmHg)	DBP (mmHg)	PP (mmHg)	Sex (0=f;1=m)	BP Group	Weight (kg)	BMI (kg/m^2)	Age (years)	PHV (years)
Dist ($mmHg^{-1}$) (n=104)	-0.03	-0.09	0.55**	-0.12	-0.12	-0.08	-0.01	-0.16	-0.13
PWV (cm/sec) (n=72)	0.59**	0.42**	0.30**	0.28**	0.46**	0.55*	0.55**	-0.11	0.07
IMT (mm) (n=105)	0.09	0.01	0.03	0.01	0.08	0.1	0.11	-0.12	-0.09

*p<0.05; **p<0.01 Dist=distensibility, PWV=pulse wave velocity, IMT=intima-media thickness, SBP=systolic blood pressure, DBP=diastolic blood pressure, PP=Finapres obtained pulse pressure, BP Group= blood pressure group, BMI=body mass index, PHV=peak height velocity.

4.4 Analysis of Covariance

According to the results of the independent t-tests, no significant differences were found for IMT or distensibility between the NBP and EBP groups. For that reason, analysis of co-variation for potential confounding factors was not required for IMT or distensibility. However, when the difference in PWV between the NBP and EBP groups was adjusted for PHV, BMI, age, and sex, a significant difference remained between groups (Table 4).

Table 4. Adjusted regression analysis for PWV and potential confounding variables between NBP and EBP ($n=72$, $r^2=0.388$)

Model	Unstandardized Regression Effect	p-value
BP Group	20.959	0.013
Sex	13.487	0.032
BMI	2.042	0.001
PHV	6.744	0.289
Age	-4.24	0.513

BP Group=blood pressure group, BMI=body mass index, PHV=peak height velocity, BP group (0=NBP, 1=EBP), Sex=% male

Chapter 5 – Discussion

To the author's knowledge, this represents the first investigation to examine the effect of BP on both central and systemic arterial characteristics in 11 to 14 year children. The major finding from this study was the significant increase in systemic PWV in children with elevated BP, with no difference in central arterial IMT or distensibility.

5.1. The Effect of Blood Pressure

5.1.1 Systemic Pulse Wave Velocity

The ability of the arterial system to cushion blood flow ejected from the heart is determined by the elasticity of the arterial walls and can be estimated by PWV (52). In an elastic arterial tree, transduction of the pulse wave throughout the body is slower compared to a stiffer arterial system. Increased PWV has been shown to be strongly correlated to atherosclerosis in both the CCA and aorta (96; 81).

The results of the current study show that children with elevated BP had significantly increased systemic arterial stiffness as measured by an 8.7% greater PWV. This difference remained after controlling for age, maturation, obesity and sex. As well, PWV was positively correlated to SBP, DBP and PP. These findings are not surprising as BP indirectly influences PWV. Chronically elevated BP leads to arterial adaptations such as stiffening of vessels, which increases pulse wave propagation speed (69). Complicating this issue further, stiffening of arterial tissue can elevate SBP and decrease DBP (69). Figure 3 illustrates the interactions between BP, PWV and arterial stiffness throughout the vascular system.

However, the PWV values reported in this study do not match those of other children studies. The reason for this discrepancy is due to the different techniques used in the literature

(11; 81; 95). The most common method is to calculate the pulse transit time between two specific arterial locations. However, the technique used in this study is less common and records the pulse transit time from the R-wave of the ECG to the upstroke of the pulse wave contour recorded at the toe (45). Thus, the reason the PWV in the present study is slower compared to other studies (11) is due to the fact that the current technique incorporates the time it takes for the heart to contract and eject the blood. In fact, the commonly used technique for obtaining PWV is to record the pulse wave between the carotid and femoral arteries (81). However, our beat-by-beat recordings of the pulse wave in the groin area of peri-pubescent children were not a viable option. Furthermore, by using a more distal location for terminal pulse wave recordings, both peripheral and central vasculature were included, which when compared with CCA distensibility may explain the possible differences in arterial stiffness that depend on proximity to the heart. Although a study that incorporated our variation of PWV in children does not exist, a publication in adult patients with a wide range of BP profiles suffering from systemic lupus (75) showed comparable results.

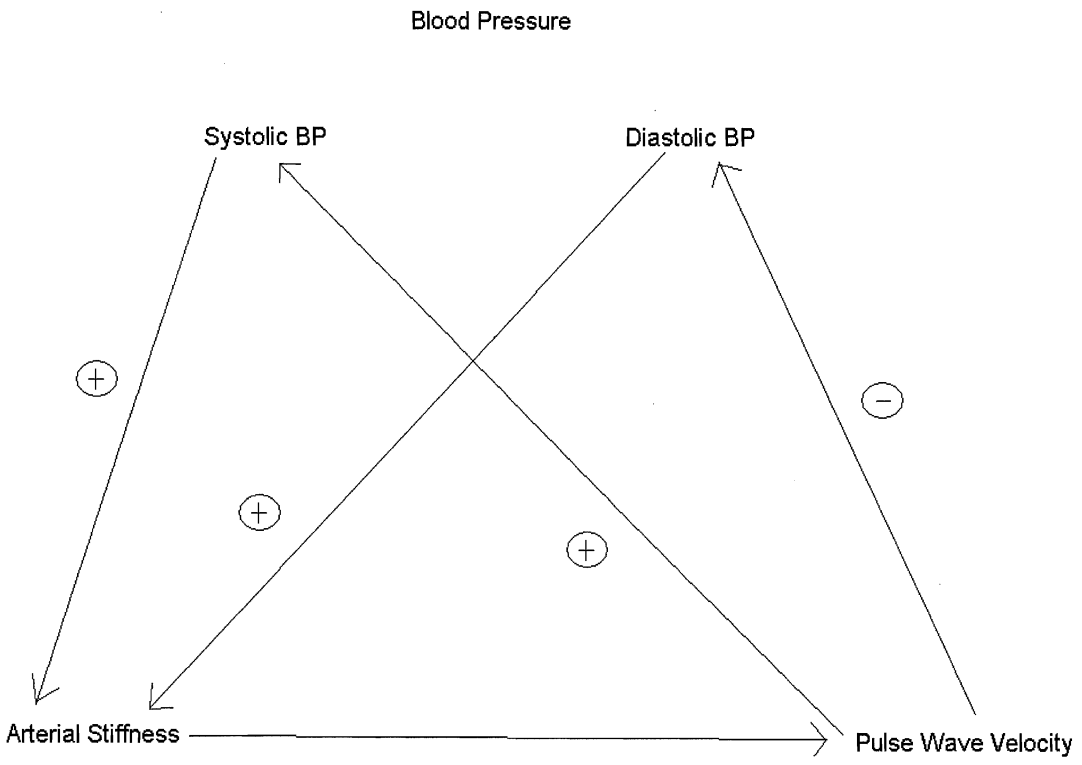

Figure 3. Diagram illustrating interactions between blood pressure and pulse wave velocity.

Nevertheless, the results of this study are in accordance with a similar investigation by Gil et al. who showed a significantly elevated brachial-ankle PWV in hypertensive adolescents aged 16-18 years (25). Likewise, Kucerova et al. showed that young normotensive adults (20-30 years old) with a parental history of hypertension had elevated PWV (carotid to femoral segment) when compared to similarly aged adults without parental history of hypertension (41). Furthermore, although both groups in Kucerova's study had BP within the normal range, the offspring of hypertensive adults had significantly elevated BP (121/75 v 114/71 mm Hg). This suggests that increases in arterial stiffness may precede chronic hypertension. The current study appears to support this theory, as the participants in this study, although significantly younger were also not clinically hypertensive and still showed altered systemic PWV. In fact, a study

investigating the association between BP and PWV in children after controlling for obesity, age, maturation and sex does not exist according to a comprehensive review of the literature (11, 25, 45, 48, 55). Although the current study did find significant correlations between PWV and BMI, weight, height, and sex (Table 3), a significant difference in PWV persisted between NBP and EBP groups after simultaneous co-variation of all these potential confounders. This implies that BP status has an independent association with peripheral arterial stiffness in children.

5.1.2 Arterial Intima-Media Thickness

In the current study, there were no significant differences in CCA IMT between the NBP and EBP groups and IMT did not correlate with SBP, DBP or PP. Common carotid artery IMT is a common estimate of arterial wall thickness that has been shown to positively correlate with atherosclerotic plaque presence (12) and predict future clinical cardiovascular events (28). According to the results of this study, the EBP group did not possess a significantly thicker arterial wall structure as compared to the NBP group. This is in contrast to that reported by Litwin and colleagues (50; 51) who showed both carotid and femoral artery IMT to be significantly elevated in their hypertensive population aged 5-20 years. The apparent discrepancy could be due to several important differences between studies. First, Litwin and colleagues (50; 51) included a wide age spectrum of individuals including infants to young adults. Duration of risk factor exposure is known to correlate with advancing atherosclerotic disease (15; 60; 64). Therefore, older subjects in Litwin's studies would have naturally been exposed to hypertension much longer than younger children, exacerbating the difference between groups in arterial markers. As well, aging itself has been shown to have a detrimental effect on arterial thickness (31; 71). Second, the BP reported in the current EBP group is relatively low. The two aforementioned Litwin publications reported SBP in the children with hypertension to be between 129 and 131

mmHg, while the SBP in the EBP group of the current study was only 110 mmHg, with the majority of our participants in the EBP group being clinically normotensive (23) . A higher SBP would likely lead to an increased IMT, taking into account that SBP is the dominant predictor of atherosclerosis (91) and has been shown to relate to IMT even when DBP does not (76). However, the current study appears to agree with a study by Stabouli and colleagues that investigated carotid IMT in adolescents with normal BP and those with white coat hypertension (aged 14 ± 4.5 years) (86). Although both groups were in the normal clinical range during 24-hour ABPM, the WCH group had significantly elevated BP as compared to the normotensive group (118/68 vs.112/65 mmHg) . As well, no difference in IMT was found between BP groups. Taking into account Stabouli's results, our study indicates that significant elevations in BP may not lead to central arterial atherosclerotic progression in the form of increased CCA IMT, so long as BP remains within the clinically accepted normal range.

5.1.3 Arterial Distensibility

Distensibility represents the change in vessel diameter for a given change in pressure (34) and estimates arterial stiffness in a localized region within the arterial vasculature; commonly the CCA (69). Common carotid artery distensibility has been shown to negatively correlate with atherosclerotic plaque severity in the CCA as well as the aorta, and predict future CVD (95). In this study, differences in CCA distensibility between the NBP (0.0064 ± 0.0019mmHg^{-1}) and EBP group (0.0058 ± 0.0024 mmHg $^{-1}$) were not significant, but are similar to that reported in previous work (2). The studies by Litwin and colleagues that report CCA distensibility in children with severe hypertension are lower than what was reported in the current study (50; 51). A possible explanation for this is that atherosclerotic progression increases with age after puberty (71), and that atherosclerosis severity increases with increasing BP (84). Also, CCA distensibility

significantly decreases in children/adolescents with increasing age cohorts (11-14 years, 14-17 years, 17-20 years) (34). In light of this, the differing CCA distensibility between this study and Litwin and colleagues (50; 51) is likely due to the wide age ranges used in their studies and the aforementioned very high SBP of the children in their hypertensive group. Similar to the IMT results, central arterial atherosclerotic progression as estimated through CCA distensibility is unaltered in our EBP children.

5.2 Physiological Implications

This study showed differing results in arterial elastic measures when using different segments of the vasculature for estimation. Systemic arterial stiffness, as measured through PWV, was reduced in the EBP group, but central arterial stiffness as measured through CCA distensibility was not. It is likely that the differences may, in part, be explained by arterial wall composition between central and peripheral arteries.

It is well established that central arteries are comprised primarily of elastin and contain relatively few muscle fibres (58). In contrast, as arteries become more peripheral the relative makeup of the arterial wall changes to become more muscular and less elastic (58). In addition, when moving from central to more peripheral arteries there is an increase in receptor density of adrenergic post-junctional alpha receptors, which respond heavily to sympathetic neurotransmitter release (90). Striimper and colleagues have described the primary method by which the microvasculature regulates blood flow as receptor activated signalling pathways that act to alter arterial diameters, in turn altering resistance to flow (89). For instance, it has been shown that the stiffness of muscular conduit vessels (i.e. brachial artery, radial artery) is increased during heightened sympathetic nerve activity such as lower body negative pressure and cold pressor test (13; 82). As well, Zamir et al. showed that the "lumped" forearm vascular bed had

decreased compliance and increased resistance during sympathetic activation (101). In the more central carotid artery however, studies have shown increased sympathetic activation having no effect on arterial stiffness (54, 88). Further, when looking at the proximal and more elastic portion of the brachial artery, Bjarnegard and colleagues showed no change in stiffness in response to lower body negative pressure induced sympathetic activation (7). Indeed this suggests that stiffness of the central arteries is less capable of being influenced by sympathetic activation than the systemic vasculature.

Since the etiology of pediatric hypertension is not fully known, a possible contributing factor to the current findings could be increased sympathetic nerve activity. In fact, emphasizing the likelihood of this interpretation is a number of adult studies demonstrating a positive relationship between BP and directly measured sympathetic activity (22; 94). As well, an analysis performed on the same participant sample (Fitzgibbon et al. unpublished) reported significantly elevated low frequency/high frequency heart rate variability ratio in the EBP group compared to the NBP group, indicating increased sympathetic outflow in the EBP cohort. Hence, a possible mechanism for the observed increase in systemic PWV could be an increased sympathetic outflow leading to peripheral vasoconstriction and reduced arterial diameter, causing an increase in overall BP (29) and increased peripheral PWV (69). In turn, since sympathetic activation has little influence on the more centrally located arteries (i.e. CCA), this explains the lack of change in CCA distensibility found in the current study. Overall, the lack of central arterial adaptations, but an increase in systemic arterial PWV in the EBP group suggests that increases in systemic arterial tone and BP precede significant central arterial adaptations. Therefore, these results shed some light on the mechanistic pathway that leads to pediatric hypertension.

5.3 Limitations and Future Considerations

Although the current study adds new knowledge to the literature, there are recognizable limitations that need to be highlighted. Firstly, BP of the EBP group was low when compared to similar studies attempting to investigate the role elevated BP plays in arterial health. In fact, although significantly elevated in comparison to the NBP group, according to the 1996 National High Blood Pressure Education Program (23) (which measured BP in over 80,000 children within the United States) the BP of the EBP group was below the 90th percentile, technically designating them as normotensive. This difference could possibly explain the lack of correlation between BP and central arterial IMT and distensibility.

Secondly, it would have been beneficial to measure PWV within specific segments of the vasculature. Measuring from the ECG to toe pulse consists of a very large vascular segment, while having several smaller portions would have allowed for a more detailed analysis of local arterial changes along the vascular tree. This in turn would have strengthened our findings allowing for a comparison of central vs. peripheral instead of central vs. systemic vasculature.

As for future studies, it would be interesting to follow the same cohort of children over time and create a time course for if and when central arterial thickness and stiffness adapt to chronically elevated BP. Having a larger sample size with higher BP in the EBP group may satisfy some of the above limitations and considerations (see power analysis calculations in Appendix 7). Although lipid profiles were measured in the current study, they were not included in the analysis. Future consideration of lipid profiles might provide a clearer picture of the potential interaction of obesity, elevated BP and arterial characteristics. In addition, future work could incorporate baroreflex function measures to clarify the role the baroreceptors have in chronic BP elevation and how this relates to arterial structure and function. Furthermore, an exciting direction would be to investigate regional arterial changes to autonomic disturbances

such as elevated sympathetic activation in children with chronic disorders such as hypertension and obesity.

5.4 Conclusions

This study showed a significant increase in systemic PWV, but no difference in central arterial IMT or distensibility in children with elevated BP aged 11 to 14 years after controlling for age, PHV, sex and BMI. This study suggests that changes in systemic arterial stiffness precedes those more centrally, and may result from sympathetic predominance in children with above normal BP.

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APPENDICES

Appendix 1

Research Ethics Board Approval

FROM: Linda Rose-Krasnor, Acting Chair
Research Ethics Board (REB)

TO: Terrance Wade, Community Health Sciences
Paul Leblanc, Deborah O'Leary

FILE: 07-060 - WADE

DATE: October 1, 2007

The Brock University Research Ethics Board has reviewed the research proposal:

Social Determinants of Child Hypertension

The Research Ethics Board finds that your proposal requires clarification: The researcher may proceed with the work as soon as the following issue(s) have been addressed and received clearance by the Board:

Section A – General Information
5 – Other Ethics Approval/Permission
· Please forward approval from the NCDSB once it has been obtained.
***This approval will be sought upon receiving Brock University Ethics approval*

Section D – The Informed Consent Process
Consent form
· Please explain the Tanner stage instrument and how it will be administered. Will parents be present?
***The Tanner Stage instrument has been removed. In its place, skeletal maturity assessment has been added. This is a non-invasive procedure using the Sunlight BonAge™ System (Sunlight Medical, Ltd, Tel Aviv, Israel) to determine skeletal age.*

This protocol has previously been approved in: REB 04-419 to Deborah O'Leary
***please see attachment entitled 'Overview of Lab Visit and Testing Procedures', body composition paragraph*

Parents will be invited to be present for all testing during the lab visit.

18 – Consent by an authorized party
· Please clarify the verbal assent process and provide a script or guideline for obtaining verbal assent.
***please see attachment entitled 'HBeat Verbal Assent Guidelines' for a detailed outline of the telephone script for obtaining verbal assent.*

***As well, please see attachment entitled 'HBeat Consent Form' for the consent form that parents and children will read and sign in the lab prior to testing that will again detail the lab tests and testing protocol.*

· Please add a statement to all participant materials that advises parents to keep a copy for their own records.

***please see attachment entitled 'Information Letter to Parents', paragraph three.*

***As well, we will provide them with a copy of the consent form at their lab visit.*

20 – Feedback to Participants

· In your application, you state that parents may access their individual child's results by contacting the researchers. This information should appear in the consent form. What explanation would accompany these results? Please make it clear to parents that results are not diagnostic.

***please see attachment entitled 'Information and Consent' for this information.*

For each point of clarification, please highlight the changes you have made on the corresponding documents.

If you would like further information or assistance in responding to these clarification requests, please contact Lori Walker (ext. 4876) in the Research Services Office.

No research with Human Participants will commence prior to receiving ethics clearance from this board.

LRK/bb

Appendix 2
Parental Take-home letter

FACULTY OF APPLIED HEALTH SCIENCES • 905.688.5550 • www.brocku.ca/fahs/

Dr. Terrance Wade • ext 4146 • twade@brocku.ca
Dr. Deborah O'Leary • ext 4339 • doleary@brocku.ca
Dr. Paul LeBlanc • ext 4216 • pleblanc@brocku.ca

Dear Parents and Guardians:

Recently you were contacted by **HBeat** (Heart Behavioural Environmental Assessment Team) regarding your child being randomly chosen to participate in the second phase of the research study to examine high blood pressure (hypertension). Thank you for volunteering to participate in the second phase of the study! As we discussed, you and your child likely already completed some questionnaires that were sent home from school for the first phase of the study. At school, your child also had their blood pressure, height, weight, hip and waist circumference measured and completed some questionnaires about their physical activity.

For this phase of the study, the testing will take place at Brock University. The total testing should take about 1½ hours. We ask that the parent/guardian is present for at least the first half-hour. Your child will receive $20 as a token of our appreciation for you and your child's participation in this phase of the study.

Although we already discussed some of this information over the phone, we ask that you read the attached information form detailing the visit and testing procedures that will be done (Overview of Lab Visit and Testing Procedures), and keep the information for your records.

Some factors such as food, exercise and temperature, may affect the lab tests. Therefore, we ask that your child does not eat or drink anything except water once they go to bed the night before testing. We will provide your child with an allergy-sensitive meal that morning. We also ask that your child avoids caffeine (for example, coffee, tea, cola) and refrains from exercise the morning of testing. Finally, your child should either be dressed in athletic attire (shorts, t-shirt, or tank top and running shoes) or bring these clothes to the testing session.

At the beginning of your visit to Brock University, we will go over the lab procedure and you will have an opportunity to ask any questions related to the tests. Once you are satisfied, we will have you and your child sign a consent form to allow your child to participate in this phase of the HBeat research study.

This study has been reviewed and approved by the Research Ethics Boards from both Brock University and the Niagara Catholic District School Board. If you have any questions about the study or lab testing procedures, please contact Dr. Deborah O'Leary at 905-688-5550 (x4339), Dr. Paul LeBlanc at 905-688-5550 (x4216), or Dr. Terrance Wade at 905-688-5550 (x4146). If you have any further questions regarding your rights as a research participant, contact the Research Ethics Officer in the Office of Research Services at (905) 688-5550 (x3035) or email at reb@brocku.ca.

We are very grateful that you and your child have agreed to take part in this important study. Thank you for your time.

Sincerely,

Terrance J. Wade, Ph.D. Deborah O'Leary, Ph.D. Paul LeBlanc, Ph.D.

[1] **Principal Investigators:** Dr. Terrance J. Wade • Dr. Deborah O'Leary • Dr. John Cairney
Co-investigators: Dr. Paul LeBlanc • Dr. Jian Liu • Dr. Colleen Hood • Dr. John Hay • Dr. Panagiota Klentrou • Dr. Brian Roy • Dr. Dawn Zinga • Dr. Kevin Shoemaker

[2] **File 06-315 WADE:** Research Services • Brock University • Room C315 • 905.688.5550 ext 4315

Appendix 3
Parental Information and Informed Consent Forms

SOCIAL DETERMINANTS OF CHILD HYPERTENSION

Your child _____ is invited to participate in a research study being conducted by the investigators listed below. Prior to participating in this study you are asked to read and sign this form, which outlines the purpose and gives a detailed description of the testing procedures used in the study. The testing procedures will be conducted at Brock University.

Primary Investigators	Department	Phone	Email
Dr. Terrance J. Wade	Community Health Sciences	(905) 688-5550 x4146	twade@brocku.ca
Dr. Deborah O'Leary	Community Health Sciences	(905) 688-5550 x4339	doleary@brocku.ca
Dr. Paul LeBlanc	Community Health Sciences	(905) 688-5550 x4216	pleblanc@brocku.ca

PURPOSE

Adults in Canada and the United States have a 90% lifetime risk of developing high blood pressure, also known as hypertension. High blood pressure (HBP) is one of the strongest predictors of heart disease. Some scientists suggest that HBP may start in childhood, but we do not know what factors may be involved. This study will help us learn what factors may contribute to childhood hypertension. If we can learn what these factors are, we might be able to offer suggestions to families, schools, and communities to improve the heart health of children. This knowledge could help all children in Canada lead healthier lives and reduce their chance of having heart disease as adults.

OVERVIEW OF LAB VISIT

Upon arrival at Brock University, you and your child will be familiarized with the laboratory and the testing procedures will be explained. Once you are satisfied with the explanation of the testing procedures, you and your child will then be asked to read the information sheet about the study and sign the consent form. If your child is not already wearing athletic attire, she/he will be asked to change in the washroom.

The testing will begin with the child's blood test using a finger prick to obtain two or three drops of blood for analysis. Following this, your child will be given an allergy sensitive breakfast before any further testing.

After they have finished breakfast, they will also be asked to empty their bladder, as this has been shown to have an effect on blood pressure. Before testing blood pressure, your child will be in a sitting position for 15 minutes. Then their blood pressure will be taken using an automatic blood pressure monitor (the same unit that was previously used in school to measure your child's blood pressure).

Next, your child's weight, height, skinfold thickness, waist circumference, hip circumference, and skeletal maturity will be recorded. Finally, the child will lie down and have her/his heart rate monitored and have their heart and right carotid artery (artery in the neck) imaged using Doppler ultrasound (the same type of ultrasound seen in a hospital). At the end of testing, your child will receive $20 as a token of our appreciation for your involvement in the study.

Parents are more than welcome to be present for testing.

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OVERVIEW OF TESTING PROCEDURES

The following procedures are described in further detail below:

Blood Analysis

Blood Pressure Measurement

Body Composition

Heart Rate

Carotid Artery and Heart Ultrasound

TESTING PROCEDURES

Blood Analysis
Finger pinprick blood sampling - The middle finger of the non-dominant hand (e.g. if right handed, the middle finger of the left hand will be used) will be pricked so two drops of blood can be sampled. They will feel the small prick but will not feel any pain or discomfort for the remainder of the sampling. The tip of that finger may feel sensitive and a little bit sore for about a day. It is important to keep the site clean and covered with an adhesive bandage until it is healed (up to 24 hours) to minimize any risk of infection.

Blood Pressure
Blood pressure is monitored using a non-invasive method. The method involves an automated arm cuff system that is similar to the method used in a doctor's office. A cuff is wrapped around the upper arm and is inflated then deflated. After sitting in a resting position for 15 minutes, the child will have their blood pressure taken automatically, 6 times in 1 minute intervals. No risk is involved.

Body Composition
All body composition measures will take place behind a portable curtain for privacy with the parent present if desired. Height, weight, hip and waist circumference will be measured. Skinfold thickness will be assessed using a non-invasive method that measures skin thickness. The tester pinches the skin at the appropriate site to raise a double layer of skin and the underlying adipose tissue, but not the muscle. The calipers are then applied at right angles to the pinch and a reading is taken. Skinfold measures will be taken at two sites including the subscapular (lower shoulder blade) and triceps (back of the upper arm). No risk is involved. Next, your child's forearm, lower leg and wrist will be measured using Doppler ultrasound to gauge skeletal maturity. The ultrasound technique used is completely non-invasive and similar to that used to visualize the development of a baby during pregnancy. Again, no risk is involved.

Heart Rate
Heart rate will be measured using sensors placed on the skin of your child's upper chest. These sensors are electrodes used to detect the electrical activity generated by the heart and do not transmit electrical signals into the body from the heart rate monitor. No risk is involved.

Carotid Artery and Heart Ultrasound
All carotid artery and heart ultrasound measures will be taken in a lying position in a private room. In addition to the ultrasound previously mentioned, two more ultrasound measures will be performed. First, the carotid artery ultrasound will be performed using a small transducer to visualize the carotid artery. As well, carotid artery blood pressure will be simultaneously obtained using a thin pen-like device that is lightly pressed against the neck. Both the probe and pen like-device will be pressed against the neck on opposite sides. It is a non-invasive procedure. Second, the ultrasound of the heart will be performed with a small probe placed between your child's ribs on the left side of their chest. This procedure is also non-invasive and no risk is involved.

POTENTIAL RISKS AND DISCOMFORTS

Please refer to the "Testing Procedures" previously mentioned for a complete description of the procedures to be performed during the study and the potential risks associated with these procedures. If an injury occurs at any time during the investigation, appropriate first aid/CPR will be administered and you will be advised to seek necessary medical help.

BENEFITS AND REMUNERATION

A potential benefit for your child's participation in this project is the knowledge of their blood pressure, heart and artery assessment, as well as any underlying cardiovascular disease risk factors. Your child will also be reimbursed $20 as a token of our appreciation for you/your child's involvement in the study.

CONFIDENTIALITY

All data collected during this study will remain confidential and stored in offices and on secured computers to which only the principal investigators, co-investigators, project coordinator, and research assistants will have access. The electronic file containing your answers and body measurements will only contain a unique identification number and no other identifying information to ensure your information is completely anonymous. You should be aware that the results of this study will be made available to the scientific community, through publication in scientific journals; however, we will only use the combined data from families so that no single child or family can be identified. You will have access to your child's data, as well as the group data when it becomes available and if you are interested. Please contact the researchers directly if you wish to obtain this information.

PARTICIPATION AND WITHDRAWAL

You can choose whether your child participates in this study or not. You may exercise the option of removing your child's data from the study if you wish. You may also refuse to answer any questions posed to you and/or your child during the study and still remain as a participant in the study. The investigators reserve the right to withdraw your child from the study if they believe that circumstances have arisen which warrant doing so.

RIGHTS OF RESEARCH PARTICIPANTS

You may withdraw your consent to participate in this study at any time, and you may also discontinue the participation of your child at any time without penalty. In signing this consent form or in participating in this study you are not waiving any legal claims or remedies. This study has been reviewed and received clearance from the Brock University Research Ethics Board (File 07-060 - WADE). If you have any further questions regarding your rights as a research participant contact the Research Ethics Officer in the Office of Research Services at (905) 688-5550 x3035 or email at reb@brocku.ca.

INFORMATION

Please contact Dr. Deborah O'Leary at 905-688-5550 (x4339), Dr. Paul LeBlanc at 905-688-5550 (x4216) or Dr. Terrance Wade at 905-688-5550 (x4146) if you have any questions about the study.

I have read and understand the above explanation of the purpose and procedures of the project and I am aware of the potential risks and my rights as a research participant. I understand that I can gain access to my child's individual results by contacting the researchers, and I understand that these results are not a clinical diagnosis. My questions have been answered to my satisfaction and I agree to allow my child to participate in this study.

CONSENT FORM	
☐ I do give permission for my child to participate in the Brock University HBeat lab component conducted by Dr. Terrance J. Wade, Dr. Deborah O'Leary and Dr. Paul LeBlanc.	
☐ I do not give permission for my child to participate in the Brock University HBeat lab component conducted by Dr. Terrance J. Wade, Dr. Deborah O'Leary and Dr. Paul LeBlanc.	
Signature of Parent/Guardian:	Date:
Printed Name of Parent/Guardian:	
Signature of Student:	Date:
Printed Name of Student:	

In addition to the current study, data collected may be used later to answer other research questions that may arise from this study. We would like your permission to keep the information that you and your child provided on file after this research study is over. All stored personal data will be kept strictly confidential and all information will be coded so that no one will be able to identify you or your child. At any time, you can ask to have your information removed and not included in any future projects by contacting Dr. Terrance J. Wade (905-688-5550 ext 4146) or Dr. Deborah O'Leary (905-688-5550 ext 4339).

☐ I do give permission to have my information and my child's information stored to use to answer future research questions after the HBeat study is over.	
☐ I do not give permission to have my information and my child's information stored to use to answer future research questions after the HBeat study is over.	
Signature of Parent/Guardian:	Date:
INVESTIGATOR	
In my judgment the participant is voluntarily and knowingly giving informed consent and possesses the legal capacity to give informed consent and participate in this research study.	
Signature of Investigator:	Date:

Appendix 4
Participant Information

LABORATORY INFORMATION SHEET

Date: _____ Time (am/pm): _____

SECTION 1: STUDENT INFORMATION			
Student ID#:	**Name:**		
Gender:	**DOB**(mmddyyyy)**:**	**Age:**	
Allergies:	**Medications:**		
Medical Concerns:			
Height: _____ **cm**	**Sitting Height:** _____ **(-74) =** _____ **cm**	**Weight:** _____ **kg**	**BMI:** _____ **(kg/m²)**

SECTION 2: QUESTIONNAIRES and CONSENTS	
STUDENT	**PARENT**
1. Consent (signed): Y N	1. Consent (signed): Y N
2. Current Med History: Y N	2. Missing Data (PQ & CQ), Sleep Questionnaire: Y N

SECTION 3: BLOOD ANALYZER	
Cholestech LDX StIcker	**Notes: (Please note any changes to protocol, problems during testing, other circumstances that would hinder test results)**

SECTION 4: BONE ULTRASOUND AND AUTOMATIC BLOOD PRESSURE

Bone age:_____ years	Note: Non-dominant arm only

Blood Pressure (SBP/DBP) **Right Arm Only (if errors occur, take an additional reading)**

2.	3.	4.	5.	6.

SECTION 5: BODY COMPOSITION MEASURES

Examiner:

Waist Circumference	**Hip Circumference**
Trial #1: _____ **cm**	Trial #1: _____ **cm**
Trial #2: _____ **cm**	Trial #2: _____ **cm**

Waist : Hip Ratio

_____ : _____ **cm**

Skinfold Measurements

Examiner:

SITE	**TRIAL 1 (mm)**	**TRIAL 2 (mm)**	**TRIAL 3 (>1mm)**	**MEDIAN (mm)**
SUBSCAPULAR				
TRICEPS				

SUM OF SKIN FOLDS: _____ **(mm)**	PERCENT BODY FAT: _____ **(%)**
1. Have you had your period?: Y N	2. How old were you when you first had your period?: _____ yrs
3. How often do you get periods?: _____ days	4. How long does your period last for?: _____ days

SECTION 6: ARTERIAL MEASUREMENTS

Doppler Settings

Examiner:

Frequency: 10.0 mHz	Power: 0 dB	Persistence: turn to minimum
Depth: _____ **cm**	FPS: change focus # (decrease to 2) to increase fps	

Blood Pressure (SBP/DBP)

Pre 1		**Post 1**	
2		**2**	
3		**3**	

Arterial Measurements

Systolic Diameter: _____ **mm**	Compliance:
Diastolic Diameter: _____ **mm**	Distensibility:
Diameter Change: _____ **mm**	CaPP: _____ **mmHg**
Baroreflex Sensitivity: _____ **ms/mmHg**	Mean HR: _____ **bpm**
Distance Measurements	
Sternal notch to toe: _____ **cm**	Sternal notch to carotid: _____ **cm**
Notes for Cardiovascular Component	

SECTION 7: LEFT VENTRICULAR MASS MEASUREMENTS	
Examiner:	
Probe: _____	Depth: _____ **cm**
B-Mode Images:	M-Mode Images:
Interventricular Septum (end-distole): _____ **cm**	Ejection Fraction: _____ **%**
Left Ventricular Diameter (end-diastole): _____ **cm**	Circumferential Fiber Shortening: _____ **%**
Left Posterior Wall (end-diastole): _____ **cm**	Stroke Volume: _____ **ml**
End Diastole Volume: _____ **ml**	Left Ventricular Mass: _____ **g**
End Systole Volume:_____ **ml**	Left Ventricle Mass Indexed by BSA: _____**g/m2**
	Left Ventricle Mass Indexed by HT: _____**g/m2.7**
Notes: Please note any changes to protocol, problems during testing, medical conditions that would hinder test results	

Appendix 5
Years from Peak Height Velocity Equations

Boys: Maturity Offset = -9.236 + 0.0002708·Leg Length and Sitting Height interaction -0.001663·Age and Leg Length interaction +0.007216·Age and Sitting Height interaction +0.02292·Weight by Height ratio, where R = 0.94, R^2 = 0.891, and SEE = 0.592.

Girls: Maturity Offset = -9.376 + 0.0001882·Leg Length and Sitting Height interaction - 0.0022·Age and Leg Length interaction +0.005841·Age and Sitting Height interaction - 0.002658·Age and Weight interaction +0.07693·Weight by Height ratio, where R=0.94, R^2=0.890,and SEE = 0.569.

Appendix 6
Statistical Output

Descriptives of NBP (0) and HBP (1) children

	hyper	N	Mean	Std. Deviation	Std. Error Mean
trueage	0	85	12.7637	.81889	.08882
	1	21	13.0564	.91345	.19933
phv	0	85	-1.7080	.80756	.08759
	1	21	-1.6800	1.04358	.22773
student gender (M/F)	0	85	.3412	.47692	.05173
	1	21	.4762	.51177	.11168
(cm)	0	85	157.9812	9.28567	1.00717
	1	21	160.5524	9.42595	2.05691
Mean of Weight Measures	0	85	46.7094	11.56612	1.25452
	1	21	65.0659	19.58840	4.27454
Body Mass Index	0	85	19.3963	3.59042	.38944
	1	21	25.9490	6.47583	1.41314
LabFieldAveS	0	85	90.7725	5.00845	.54324
	1	21	110.1429	6.11198	1.33374
LabFieldAveD	0	85	55.1431	4.77857	.51831
	1	21	70.8810	5.38443	1.17498
LabFieldMAP	0	85	67.0196	4.38267	.47537
	1	21	83.9683	4.84973	1.05830

	hyper	N	Mean	Std. Deviation	Std. Error Mean
sdave	0	83	5.4319	.43048	.04725
	1	21	5.6011	.38638	.08431
ddave	0	83	4.8256	.43039	.04724
	1	21	4.9434	.39280	.08572
diamdiff	0	83	.6063	.10947	.01202
	1	21	.6124	.10959	.02392
DistwFINApresPP	0	83	.006397623	.0018895849	.0002074089
	1	21	.005820455	.0024317884	.0005306597
FinapresAdjPP	0	83	44.2750	10.76881	1.18203
	1	21	51.7680	12.49781	2.72725
pwv	0	56	389.4854	24.20355	3.23434
	1	15	423.1360	35.07687	9.05681
imt	0	83	.4192	.05744	.00631
	1	21	.4304	.05085	.01110

Independent T-test for children between NBP (0) and HBP (1)

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
trueage	Equal variances assumed	.694	.407	-1.433	104	.155	-.29262	.20419	-.69753	.11229
	Equal variances not assumed			-1.341	28.463	.191	-.29262	.21823	-.73931	.15407
phv	Equal variances assumed	3.291	.073	-.134	104	.894	-.02797	.20909	-.44259	.38666
	Equal variances not assumed			-.115	26.219	.910	-.02797	.24399	-.52930	.47336
student gender (M/F)	Equal variances assumed	2.196	.141	-1.145	104	.255	-.13501	.11790	-.36881	.09879
	Equal variances not assumed			-1.097	29.183	.282	-.13501	.12308	-.38666	.11663
(cm)	Equal variances assumed	.150	.700	-1.133	104	.260	-2.57120	2.26942	-7.07155	1.92914
	Equal variances not assumed			-1.123	30.325	.270	-2.57120	2.29026	-7.24643	2.10403
Mean of Weight Measures	Equal variances assumed	6.696	.011	-5.586	104	.000	-18.35646	3.28607	-24.87286	-11.84006
	Equal variances not assumed			-4.121	23.552	.000	-18.35646	4.45483	-27.56004	-9.15288
Body Mass Index	Equal variances assumed	17.983	.000	-6.256	104	.000	-6.55272	1.04748	-8.62991	-4.47552
	Equal variances not assumed			-4.470	23.121	.000	-6.55272	1.46582	-9.58412	-3.52132
LabFieldAveS	Equal variances assumed	.613	.435	-15.173	104	.000	-19.37031	1.27662	-21.90189	-16.83872
	Equal variances not assumed			-13.450	27.009	.000	-19.37031	1.44013	-22.32517	-16.41545
LabFieldAveD	Equal variances assumed	.053	.819	-13.178	104	.000	-15.73782	1.19429	-18.10614	-13.36949
	Equal variances not assumed			-12.255	28.286	.000	-15.73782	1.28422	-18.36722	-13.10841

LabFieldMAP	Equal variances assumed	.004	.949	-15.538	104	.000	-16.94865	1.09081	-19.11177	-14.78552
	Equal variances not assumed			-14.609	28.607	.000	-16.94865	1.16016	-19.32285	-14.57444

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
sdave	Equal variances assumed	.564	.454	-1.641	102	.104	-.16918	.10313	-.37374	.03537
	Equal variances not assumed			-1.750	33.724	.089	-.16918	.09665	-.36566	.02730
ddave	Equal variances assumed	.372	.543	-1.139	102	.257	-.11780	.10339	-.32288	.08728
	Equal variances not assumed			-1.204	33.247	.237	-.11780	.09787	-.31687	.08127
diamdiff	Equal variances assumed	.002	.965	-.229	102	.819	-.00613	.02675	-.05918	.04692
	Equal variances not assumed			-.229	30.893	.820	-.00613	.02676	-.06073	.04846
DistwFINApresPP	Equal variances assumed	1.392	.241	1.177	102	.242	.000577 1682	.000490 3635	-.000395 4653	.001549 8018
	Equal variances not assumed			1.013	26.427	.320	.000577 1682	.000569 7527	-.000593 0552	.001747 3917
FinapresAdjPP	Equal variances assumed	.621	.432	-2.756	102	.007	-7.49294	2.71847	-12.88502	-2.10086
	Equal variances not assumed			-2.521	27.979	.018	-7.49294	2.97238	-13.58180	-1.40408
pwv	Equal variances assumed	2.315	.133	-4.324	69	.000	-33.65061	7.78263	-49.17653	-18.12468
	Equal variances not assumed			-3.499	17.725	.003	-33.65061	9.61700	-53.87765	-13.42356
imt	Equal variances assumed	.113	.738	-.820	102	.414	-.01126	.01373	-.03849	.01598
	Equal variances not assumed			-.882	34.134	.384	-.01126	.01276	-.03719	.01468

Correlations – Entire Cohort

		SBP	DBP	FingerPP	gender	BP Group	(kg)	bmi	age	phv	Dist	imt	pwv
LabFieldAveS	Pearson Correlation	1.000	.843**	.297**	.142	.830**	.678**	.660**	.199*	.108	-.034	.089	.589**
	Sig. (2-tailed)		.000	.002	.151	.000	.000	.000	.043	.273	.733	.371	.000
	N	104	104	104	104	104	104	104	104	104	104	104	71
LabFieldAveD	Pearson Correlation	.843**	1.000	.231*	.053	.791**	.490**	.504**	.078	.021	-.090	.006	.419**
	Sig. (2-tailed)	.000		.019	.592	.000	.000	.000	.429	.831	.366	.956	.000
	N	104	104	104	104	104	104	104	104	104	104	104	71
FinapresAdjPP	Pearson Correlation	.297**	.231*	1.000	.234*	.263**	.362**	.276**	.169	.173	-.552**	.032	.307**
	Sig. (2-tailed)	.002	.019		.017	.007	.000	.005	.087	.079	.000	.747	.009
	N	104	104	104	104	104	104	104	104	104	104	104	71
student gender (M/F)	Pearson Correlation	.142	.053	.234*	1.000	.116	.161	.061	.236*	.156	-.119	.007	.273*
	Sig. (2-tailed)	.151	.592	.017		.242	.103	.537	.016	.115	.230	.948	.021
	N	104	104	104	104	104	104	104	104	104	104	104	71
hyper	Pearson Correlation	.830**	.791**	.263**	.116	1.000	.475**	.518**	.137	.009	-.116	.081	.462**
	Sig. (2-tailed)	.000	.000	.007	.242		.000	.000	.166	.927	.242	.414	.000
	N	104	104	104	104	104	104	104	104	104	104	104	71
(kg)	Pearson Correlation	.678**	.490**	.362**	.161	.475**	1.000	.933**	.293**	.216*	-.077	.099	.557**
	Sig. (2-tailed)	.000	.000	.000	.103	.000		.000	.003	.028	.435	.316	.000
	N	104	104	104	104	104	104	104	104	104	104	104	71
bmi	Pearson Correlation	.660**	.504**	.276**	.061	.518**	.933**	1.000	.101	-.054	-.013	.109	.523**
	Sig. (2-tailed)	.000	.000	.005	.537	.000	.000		.307	.583	.894	.269	.000
	N	104	104	104	104	104	104	104	104	104	104	104	71
trueage	Pearson Correlation	.199*	.078	.169	.236*	.137	.293**	.101	1.000	.743**	-.155	-.120	.103
	Sig. (2-tailed)	.043	.429	.087	.016	.166	.003	.307		.000	.116	.223	.392
	N	104	104	104	104	104	104	104	104	104	104	104	71
phv	Pearson Correlation	.108	.021	.173	.156	.009	.216*	-.054	.743**	1.000	-.131	-.093	.058
	Sig. (2-tailed)	.273	.831	.079	.115	.927	.028	.583	.000		.185	.346	.632
	N	104	104	104	104	104	104	104	104	104	104	104	71

DistwFINAprPP	Pearson Correlation	-.034	-.090	-.552**	-.119	-.116	-.077	-.013	-.155	-.131	1.000	.126	-.188
	Sig. (2-tailed)	.733	.366	.000	.230	.242	.435	.894	.116	.185		.204	.116
	N	104	104	104	104	104	104	104	104	104	104	104	71
imt	Pearson Correlation	.089	.006	.032	.007	.081	.099	.109	-.120	-.093	.126	1.000	-.011
	Sig. (2-tailed)	.371	.956	.747	.948	.414	.316	.269	.223	.346	.204		.930
	N	104	104	104	104	104	104	104	104	104	104	104	71
pwv	Pearson Correlation	.589**	.419**	.307**	.273*	.462**	.557**	.523**	.103	.058	-.188	-.011	1.000
	Sig. (2-tailed)	.000	.000	.009	.021	.000	.000	.000	.392	.632	.116	.930	
	N	71	71	71	71	71	71	71	71	71	71	71	71

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Regression Analysis – Pulse Wave Velocity

Variables Entered/Removed[b]

Model	Variables Entered	Variables Removed	Method
1	trueage, bmi, student gender (M/F), hyper, phv[a]	.	Enter

a. All requested variables entered.

b. Dependent Variable: pwv

Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.623[a]	.388	.341	24.22978

a. Predictors: (Constant), trueage, bmi, student gender (M/F), hyper, phv

b. Dependent Variable: pwv

ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	24523.187	5	4904.637	8.354	.000[a]
	Residual	38747.438	66	587.082		
	Total	63270.625	71			

a. Predictors: (Constant), trueage, bmi, student gender (M/F), hyper, phv

b. Dependent Variable: pwv

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	409.502	90.520		4.524	.00
	hyper	20.959	8.177	.287	2.563	.01
	student gender (M/F)	13.487	6.172	.214	2.185	.03
	bmi	2.042	.600	.381	3.404	.00
	phv	6.744	6.304	.186	1.070	.28
	trueage	-4.240	6.440	-.114	-.658	.51

a. Dependent Variable: pwv

Residuals Statistics[a]

	Minimum	Maximum	Mean	Std. Deviation	N
Predicted Value	371.2146	467.2689	396.3051	18.58486	72
Residual	-56.72359	61.77820	.00000	23.36105	72
Std. Predicted Value	-1.350	3.818	.000	1.000	72
Std. Residual	-2.341	2.550	.000	.964	72

a. Dependent Variable: pwv

Reliability Analysis

- Intima-media thickness measures

Intraclass Correlation Coefficient

	Intraclass Correlation[a]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.577[b]	.394	.764	9.191	19	95	.000
Average Measures	.891[c]	.796	.951	9.191	19	95	.000

Two-way mixed effects model where people effects are random and measures effects are fixed.

a. Type C intraclass correlation coefficients using a consistency definition-the between-measure variance is excluded from the denominator variance.

b. The estimator is the same, whether the interaction effect is present or not.

c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

-Diastolic diameter measures

Intraclass Correlation Coefficient

	Intraclass Correlation[a]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.913[b]	.794	.965	22.020	19	19	.000
Average Measures	.955[c]	.885	.982	22.020	19	19	.000

Two-way mixed effects model where people effects are random and measures effects are fixed.

a. Type C intraclass correlation coefficients using a consistency definition-the between-measure variance is excluded from the denominator variance.

b. The estimator is the same, whether the interaction effect is present or not.

c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

-Systolic diameter measures

Intraclass Correlation Coefficient

	Intraclass Correlation[a]	95% Confidence Interval		F Test with True Value 0			
		Lower Bound	Upper Bound	Value	df1	df2	Sig
Single Measures	.976[b]	.939	.990	80.931	19	19	.000
Average Measures	.988[c]	.969	.995	80.931	19	19	.000

Two-way mixed effects model where people effects are random and measures effects are fixed.

a. Type C intraclass correlation coefficients using a consistency definition-the between-measure variance is excluded from the denominator variance.

b. The estimator is the same, whether the interaction effect is present or not.

c. This estimate is computed assuming the interaction effect is absent, because it is not estimable otherwise.

Appendix 7
Post-hoc power analysis

The following sample size figures have been selected to proved valid inferences of required sample size for a given power.The formula used for calculating power and sample size n is:

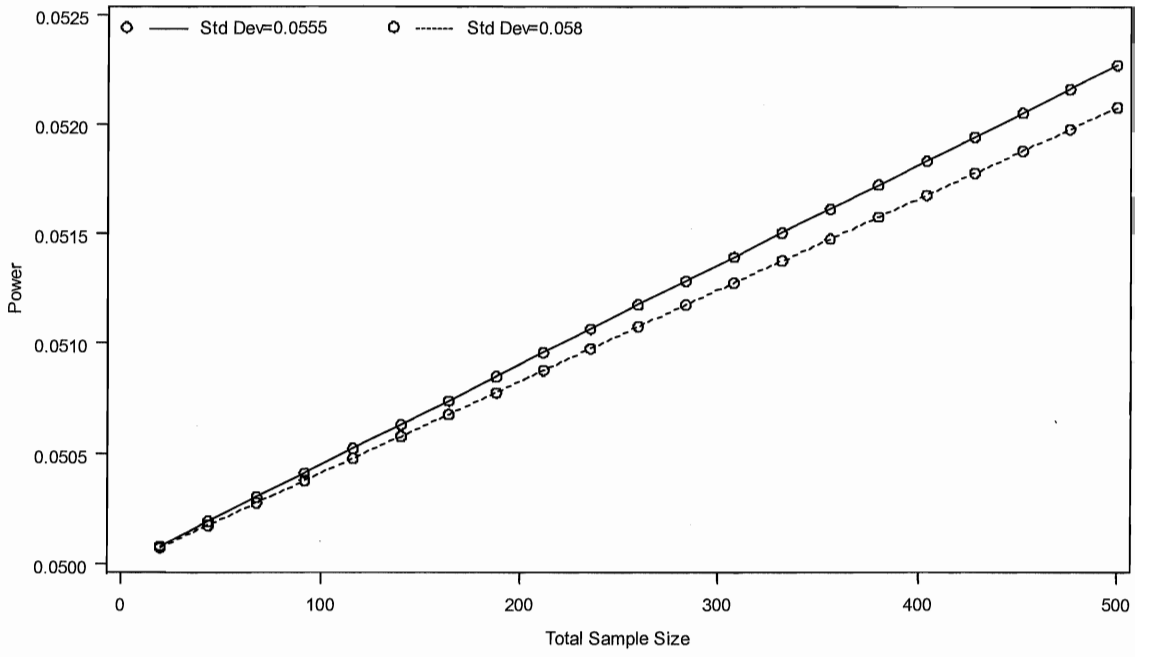
$$n = \frac{(r+1)}{r} \frac{\sigma^2 (Z_{power} + Z_{\alpha/2})^2}{(\mu_x - \mu_y)^2}$$

$$Z_{power} = \frac{\mu_x - \mu_y}{\sigma} \sqrt{\frac{nr}{r+1}} - Z_{\alpha/2}$$

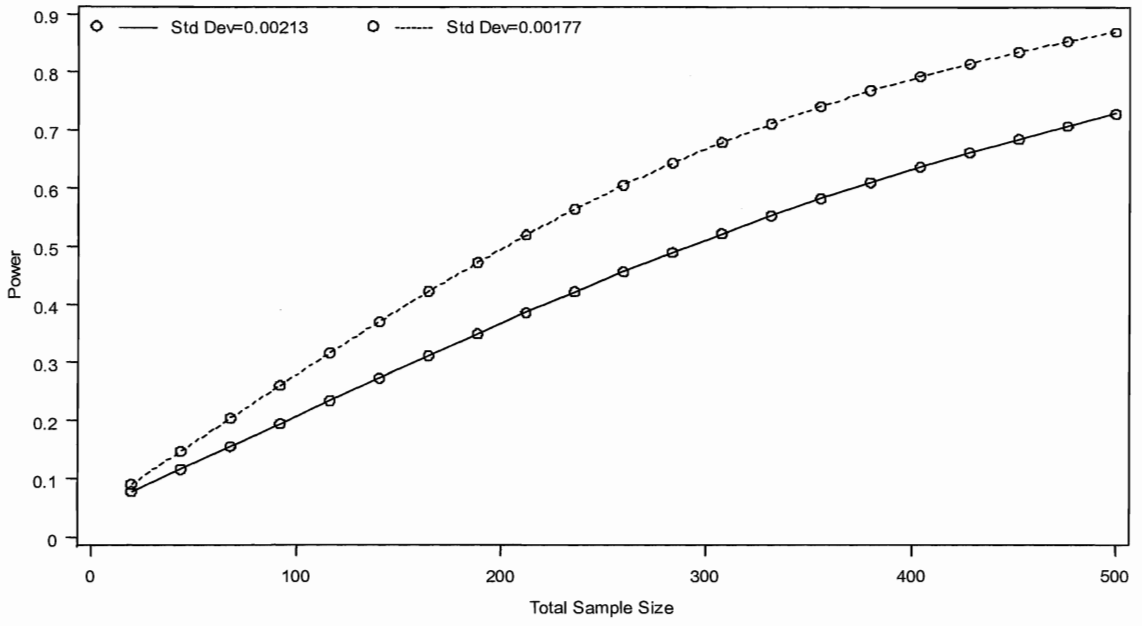
Determining Sample Size: Balancing Power, Precision, and Practicality (2008). Patrick Dattalo, Oxford University Press

For the above formulas r was the ratio of larger group to smaller group.

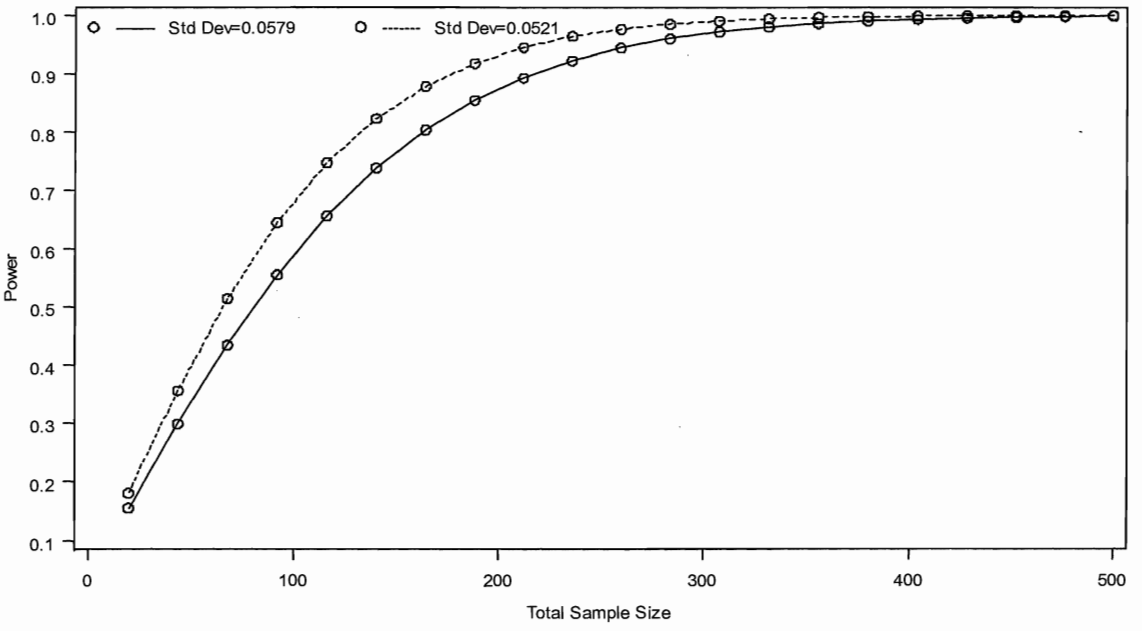
Intima-media thickness between Genders



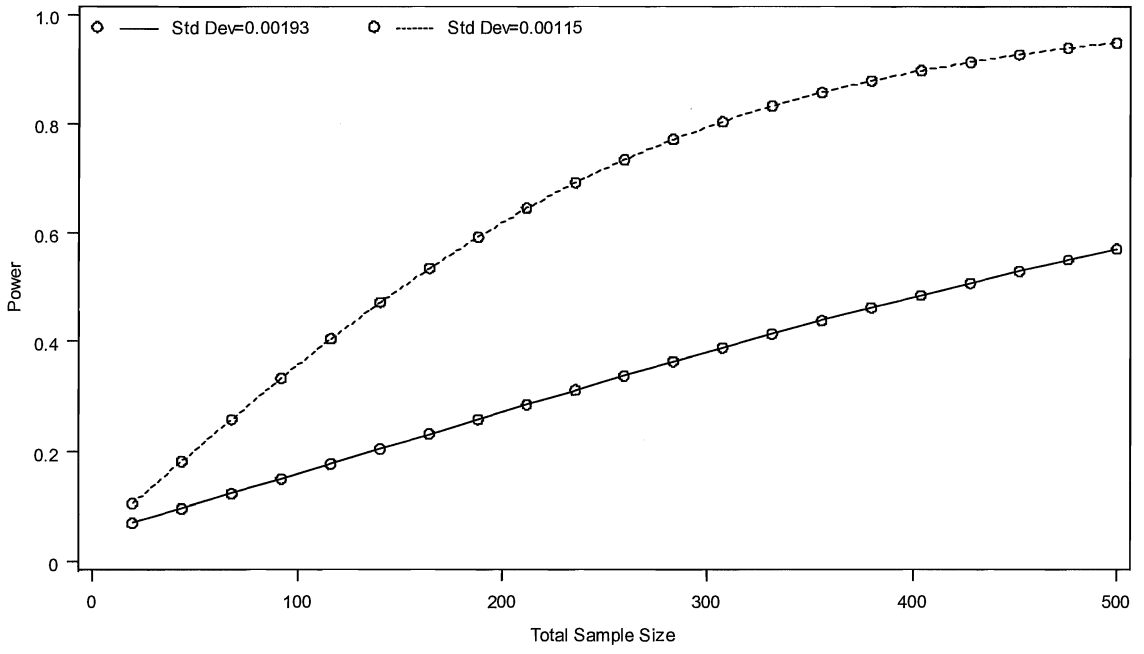
Distensibility between Genders



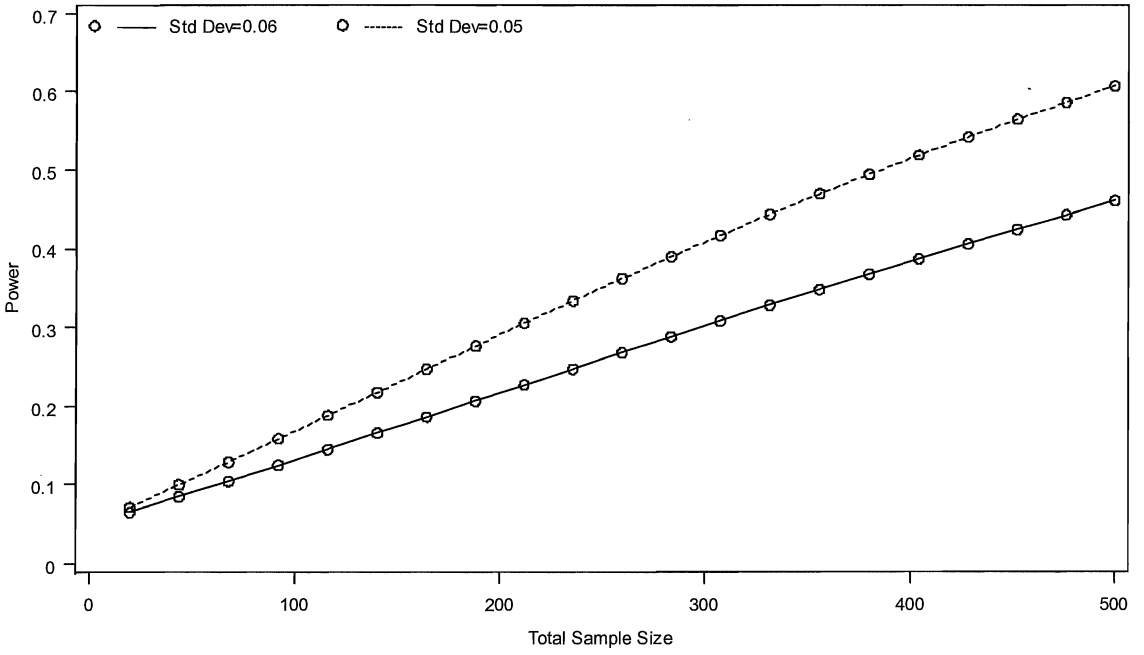
Intima-media thickness between obese and non-obese



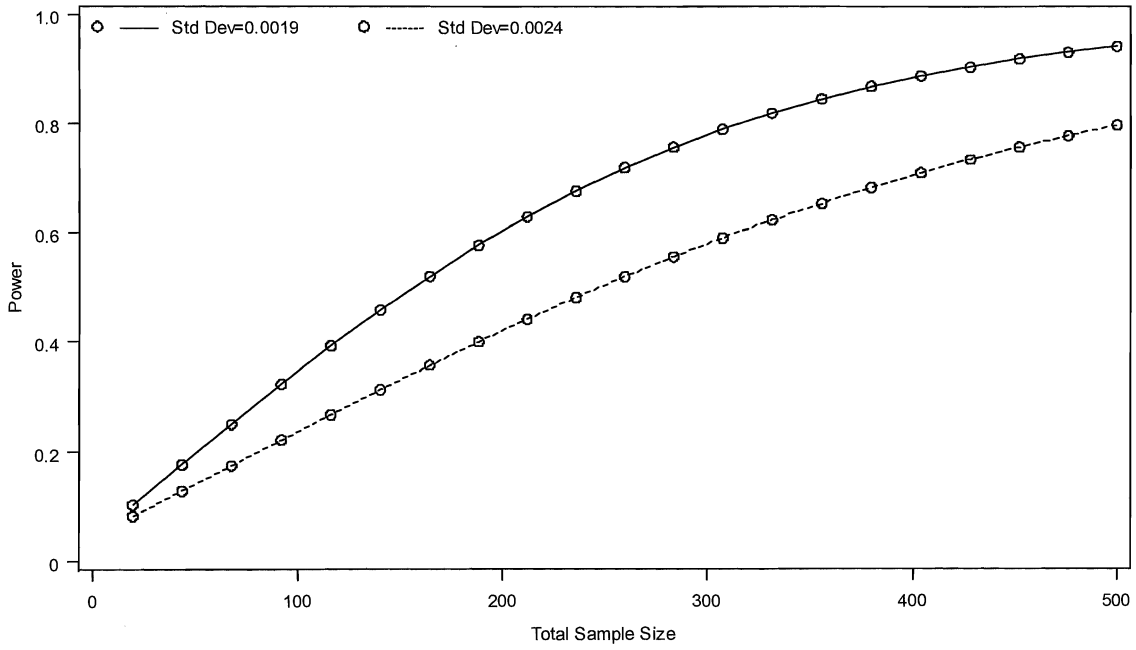
Distensibility between obese and non-obese



Intima-media thickness between BP groups



Distensibility between BP groups



Appendix 7

Raw Data

serialno	Group	LabFieldAveS	LabFieldAveD	LabFieldMAP	labasbp	labadbp	labamap	FinapresAdjPP	trueage	gender	lheight	lweight
A1048	0	92.5	52.67	65.94	95.22	53.48	67.39	41.74	11.85	0	150	39.4
A1067	0	87.17	46	59.72	109.04	63.3	78.55	45.74	11.65	0	149.3	42.4
A1127	0	87.67	51.83	63.78	101.93	52.96	69.28	48.97	12.7	0	161	42.9
A1138	0	84.5	48.67	60.61	95.49	53.98	67.82	41.5	13.85	0	149.1	36.1
A1141	0	93.67	57.17	69.33	106.46	72.2	83.62	34.26	14.01	0	160.2	66
A1291	0	91.17	59.33	69.94	103.57	80.71	88.33	22.86	14.34	0	169.1	52.2
A1365	0	93.5	57	69.17	89.6	54.04	65.89	35.56	13.33	0	156.1	40.6
A1408	0	92	53.17	66.11	125.63	71.54	89.57	54.09	12.63	0	166.8	49.6
A1441	0	86	54.67	65.11	103.66	66.01	78.56	37.64	13.14	0	155.5	50.8
A148	0	97.67	57.67	71	98.39	64.14	75.56	34.25	12.67	0	151.3	40.7
A1497	1	113.5	72.33	86.06	124.04	54.58	77.73	69.46	12.51	0	157.4	90.3
A1543	0	88.33	47.83	61.33	104.17	52.05	69.42	52.12	13.56	1	173.8	51.7
A1565	0	94.67	61.33	72.44	95.36	59.17	71.23	36.19	12.3	0	155.5	40.2
A1570	0	92.83	56.83	68.83	102.55	63.83	76.73	38.72	12.28	0	159.8	63.9
A1602	0	86.83	49.17	61.72	115.63	63.11	80.61	52.52	12.2	0	151.5	47.6
A1622	0	97.5	54.83	69.06	109.05	63.1	78.42	45.94	13.74	1	155.9	56.6
A1673	0	95.67	48.5	64.22	114.11	58.92	77.32	55.19	13.76	1	164.7	49.9
A281	1	107.17	69.83	82.28	109.35	67.76	81.62	41.59	12.81	0	149.7	38.7
A329	0	99.5	60.5	73.5	119.5	58.45	78.8	61.04	11.77	0	168.3	68.8
A490	0	89.83	51.67	64.39	112.81	54.89	74.19	57.92	12.14	1	157	45.1
A492	0	88.67	55	66.22	109.02	66.64	80.77	42.37	12.13	0	157.2	49.1
A609	0	93.83	58.5	70.28	111.87	61.42	78.24	50.45	12.56	0	159.6	53
A61	0	86.67	51	62.89	95.14	57.01	69.72	38.14	11.91	0	150.8	34.5
A661	1	109.67	76.17	87.33	137.28	73.04	94.46	64.24	14.35	1	170.5	68.2
A687	0	81.67	53	62.56	91.98	50.87	64.58	41.11	12.44	1	155.9	43.7
A693	0	86.67	52.67	64	111.59	52.39	72.12	59.2	12.17	1	163	49.9
A728	1	115.33	74.33	88	115.94	60.13	78.73	55.81	12.62	1	167.5	48
A759	0	88.5	55.17	66.28	100.57	48.68	65.97	51.89	13.26	0	167.5	57.8
A871	0	90.5	54	66.17	104.49	50.21	68.31	54.27	12.97	0	161	42.3
A872	1	98.83	64	75.61	113.26	60.28	77.94	52.98	13.76	1	145.2	38.5
A886	0	85.5	55.67	65.61	102.37	63.17	76.24	39.2	12.95	0	145.7	36.4
A890	0	97.67	62.83	74.44	92.35	57.25	68.95	35.11	13.25	0	154.9	50.9
B1078	1	108.17	65.5	79.72	112.73	62.77	79.42	49.96	12.05	0	158.4	61.1

B1090	0	82.33	47.67	59.22	84.14	51.9	62.65	32.24	11.57	0	139.9	37.6
B1099	0	88.33	61.33	70.33	98.3	65.14	76.2	33.16	12.34	1	157.6	46
B1128	0	93	61	71.67	109.37	63.64	78.88	45.73	12.96	0	163.6	39.8
B113	0	94.5	61.33	72.39	121.7	65.32	84.11	56.38	13.98	0	168.7	59.2
B1180	1	106	76.83	86.56	112.74	68.45	83.22	44.29	14.17	0	167.5	83.1
B1211	0	92.67	59.17	70.33	84.49	60.23	68.32	24.26	12.53	0	134	28.5
B1257	0	88.33	50	62.78	95.66	57.58	70.27	38.07	12.07	0	154.4	50.3
B1259	0	106	63.5	77.67	110.31	60	76.77	50.31	12.22	1	173.5	87.6
B1389	0	82.17	55.17	64.17	123.59	55.16	77.97	68.43	12.35	1	150.9	37.5
B1421	0	94.67	47.83	63.44	112.61	68.39	83.13	44.21	13.47	0	165.4	60.5
B1544	0	96.83	64.17	75.06	105.28	67.6	80.16	37.68	13.08	0	148.6	43.8
B159	0	97.33	59.83	72.33	123.93	68.07	86.69	55.86	12.4	1	165.7	63.6
B160	1	109.5	75	86.5	96.17	60.06	72.1	36.1	11.65	0	155.3	60.2
B1618	0	96.33	64.5	75.11	118.2	67.07	84.11	51.13	12.87	0	157.7	77.3
B1651	0	89	53.5	65.33	102.21	67.3	78.94	34.91	11.88	0	153.5	59
B1680	0	88.5	51.83	64.06	91.23	54.02	66.42	37.21	12.13	0	137.6	35.9
B1688	0	92.83	57.67	69.39	88.1	51.27	63.55	36.82	11.96	0	162.5	50.1
B1694	0	88	58	68	94.54	49.29	64.38	45.25	11.8	1	154.5	37
B1696	0	88.17	51.17	63.5	101.23	65.51	77.42	35.71	12.05	0	146	34.4
B173	0	88.5	50	62.83	87.59	41.58	56.92	46	13.79	0	172.3	50.1
B197	0	84.67	50.17	61.67	107.84	58.15	74.71	49.68	14.12	1	174.5	60.8
B221	0	90.67	55.17	67	99.18	45.03	63.08	54.15	12.96	0	159.3	61.1
B240	1	111.83	69.33	83.5	128.59	62.68	84.65	65.91	13.58	1	164.2	67.5
B288	0	84.67	51.33	62.44	92.67	59.04	70.25	33.63	11.43	0	134.2	29.2
B344	0	95.67	53.83	67.78	134.52	62.08	86.23	72.43	11.76	1	147.8	42.5
B568	0	91.17	57.83	68.94	102.16	70.88	81.31	31.27	12.2	1	171.8	47
B576	0	86.67	46.83	60.11	86.56	52.5	63.85	34.06	11.86	1	147.8	36.8
B579	0	94.67	61	72.22	101.51	72.27	82.02	29.24	11.75	0	168.2	60.2
B645	0	106	62.17	76.78	104.57	46.85	66.09	57.72	13.42	1	171.4	94
B669	0	84.83	50	61.61	85.68	49.78	61.75	35.9	12.45	0	155	40
B69	1	108.83	65	79.61	98.45	56.04	70.18	42.41	13.52	0	168.8	59.9
B728	0	88	47.17	60.78	83.16	62.34	69.28	20.81	11.48	0	154.3	59.7
B878	0	98.5	56.33	70.39	113.58	65.7	81.66	47.88	14.12	1	167.2	57.8
B899	1	112.67	66.67	82	139.37	94.91	109.73	44.46	13.47	1	160.9	93.5

B904	0	86.5	56.83	66.72	69.02	41.76	50.84	27.26	13.07	1	162.2	49.1
B910	0	97.5	63.5	74.83	99.49	60.65	73.6	38.84	13.2	0	167.4	55.1
B921	0	91.17	61.17	71.17	99.47	57.39	71.42	42.08	12.22	1	167	53.9
B937	0	98	53.5	68.33	116.02	61.31	79.55	54.71	13.95	1	164.3	54.1
B944	0	88.33	52	64.11	128.54	61.63	83.93	66.9	12.89	0	158.7	62.8
B957	0	88.33	61.67	70.56	126.31	63.95	84.74	62.36	13.22	0	167.8	54.4
B980	0	90.67	55.17	67	88.97	45.04	59.68	43.92	12.29	0	155	41.1
B992	0	90	53.33	65.56	117.25	71.18	86.54	46.08	12.28	1	160.7	54
B994	0	84.67	54.67	64.67	90.77	53.41	65.87	37.36	11.21	0	137.6	38.6
C115	0	88	47.83	61.22	92.04	36.41	54.95	55.63	14.32	1	167.6	80.1
C139	1	105.17	68.67	80.83	122.12	60	80.71	62.11	13.77	1	165.7	60.4
C22	0	89.67	55.67	67	95.81	58.23	70.75	37.58	14.31	1	161.3	48.2
C7	0	89.17	49.33	62.61	112.45	57.85	76.05	54.6	13.99	1	168	46.6
D108	0	94	58.17	70.11	93.04	55.24	67.84	37.8	12.01	0	153.5	48
D1116	1	108.33	69	82.11	100.43	61.09	74.21	39.33	13.13	1	150.3	67.6
D266	0	87	46	59.67	97.29	56.36	70	40.93	14.08	0	166.3	45.7
D300	1	106.67	58.17	74.33	97.4	58.67	71.58	38.73	14.49	1	165.5	67
D534	1	125.33	72.5	90.11	122.4	68.3	86.3	54.1	14.09	1	172.4	124.6
D686	0	89.5	57.67	68.28	85.8	57.2	66.73	28.6	12.51	1	150.7	47.1
D704	0	84.83	53.17	63.72	89.81	38.8	55.8	51.02	12.52	0	155	47
E1137	1	107.33	72.17	83.89	101.84	55.48	70.93	46.36	12.54	0	154.7	48.6
E199	0	91.83	58.83	69.83	118.16	60.81	79.93	57.35	13.22	0	157.9	55.9
E231	0	94.67	57	69.56	91.75	51.65	65.02	40.09	12.87	0	158	50.3
E236	1	114.17	69.33	84.28	128.31	72.27	90.95	56.04	12.81	0	164.1	100.1
E307	1	100.17	72.33	81.61	101.35	48.52	66.13	52.82	11.58	0	142.3	43
E315	0	90.67	59.33	69.78	105.73	52.73	70.4	53	11.58	0	146.9	47.7
E396	1	119.17	81.67	94.17	153.11	88.15	109.81	64.95	13.51	1	177.1	66
E442	0	90.17	53.17	65.5	108.76	65.11	79.66	43.65	13.36	1	161.6	43
E443	0	83	49.67	60.78	91.42	54.16	66.58	37.26	13.02	0	152.3	32.5
E544	0	87.17	55.33	65.94	103.59	71.85	82.43	31.74	14.33	0	167.7	46
E635	1	117.67	78.5	91.56	82.7	54.74	64.06	27.96	11.87	0	149	69
E639	0	84.83	57.83	66.83	93	59.89	70.93	33.11	11.62	1	138.5	33
E708	0	99	57.33	71.22	108.7	66.48	80.55	42.22	13.33	0	169.1	50.9
E828	1	107.5	71.17	83.28	133.59	56.09	81.92	77.5	11.9	0	165.1	85.4

E871	0	87.83	59	68.61	112.98	54.05	73.69	58.93	13.68	0	158.9	47
E876	0	97.83	60.83	73.17	104.83	51.29	69.14	53.54	13.54	0	156	47.1
E927	0	92.33	59.5	70.44	111.06	60.95	77.65	50.11	12.61	1	151.2	39.6

serialno	bmi	obese	phv	sdave	ddave	diamdiff	scsa	dcsa	pwvtime	DistwFINApresPP	imt	pwv
A1048	17.51	0	-2.18	5.29	4.69	0.6	21.99	17.29	0.33	0.0065197	0.46	380.78
A1067	19.02	0	-2.65	5.57	5	0.57	24.35	19.62	0.34	0.0052584	0.37	375.22
A1127	16.55	0	-1.29	4.78	4.25	0.53	17.94	14.16	0.32	0.0054382	0.35	415.12
A1138	16.24	0	-1.04	5.29	4.91	0.38	21.96	18.93	0.32	0.003858	0.4	381.95
A1141	25.72	1	-1.34	5.22	4.72	0.5	21.36	17.47	0.37	0.0065072	0.35	365.62
A1291	18.26	0	-0.22	5.5	4.91	0.59	23.76	18.97	0.38	0.0110452	0.4	370.85
A1365	16.66	0	-1.2	5.32	4.65	0.67	22.23	17	0.35	0.0086525	0.44	358.15
A1408	17.83	0	-1.1	4.96	4.3	0.65	19.3	14.54	0.36	0.0060563	0.45	393.24
A1441	21.01	0	-1.68	5.56	4.85	0.71	24.27	18.48	0.33	0.0083258	0.42	399.82
A148	17.78	0	-1.82	4.73	4.11	0.62	17.59	13.28	0.33	0.0094921	0.39	380.6
A1497	36.45	2	-2.98	5.73	4.15	0.58	25.78	13.51	0.32	0.0130811	0.52	424.68
A1543	17.12	0	-0.45	5.86	5.1	0.76	26.95	20.39	0.35	0.0061754	0.47	430.64
A1565	16.63	0	-1.62	5.04	4.18	0.86	19.94	13.74	0.34	0.0124611	0.48	372.32
A1570	25.02	1	-2.23	5.45	4.78	0.67	23.32	17.94	0.33	0.0077573	0.36	406.06
A1602	20.74	0	-2.33	5.34	4.68	0.66	22.41	17.18	0.35	0.0057952	0.55	373.37
A1622	23.29	1	-1.36	5.57	4.89	0.68	24.35	18.76	0.33	0.0064842	0.31	393.58
A1673	18.4	0	-0.91	4.92	4.16	0.76	19.03	13.62	0.33	0.0071939	0.57	412.12
A281	17.27	0	-1.64	5.28	4.82	0.46	21.9	18.24	0.32	0.0048168	0.48	397.33
A329	24.29	1	-2.16	5.91	5.16	0.74	27.39	20.94	0.37	0.0050483	0.48	410.9
A490	18.3	0	-1.92	4.29	3.71	0.57	14.42	10.84	0.33	0.0057068	0.45	387.04
A492	19.87	0	-2.06	4.81	4.18	0.63	18.16	13.7	0.35	0.0076838	0.41	387.62
A609	20.81	0	-1.65	5.46	4.84	0.62	23.42	18.38	0.34	0.0054344	0.44	398.34
A61	15.17	0	-2.21	4.94	4.31	0.63	19.14	14.59	0.33	0.0081837	0.43	384.5
A661	23.46	0	0.65	5.34	4.65	0.73	22.37	16.99	0.33	0.0049324	0.39	423.42
A687	17.98	0	-1.96	5.76	5.21	0.55	26.08	21.3	0.32	0.0054568	0.37	409.63
A693	18.78	0	-1.71	6.05	5.47	0.58	28.7	23.46	0.32	0.0037703	0.37	434.1
A728	17.11	0	-1.66	5.49	4.76	0.73	23.7	17.82		0.0059115	0.37	
A759	20.6	0	-0.98	5.12	4.68	0.43	20.55	17.21		0.0037383	0.54	
A871	16.32	0	-1.14	4.93	4.3	0.63	19.09	14.51	0.35	0.0058158	0.46	384.62
A872	18.26	0	-2.4	5.39	4.94	0.45	22.79	19.17	0.29	0.00357	0.39	417.46
A886	17.15	0	-1.92	4.72	4.19	0.53	17.5	13.79	0.32	0.0068767	0.45	374.67
A890	21.21	0	-1.53	5.09	4.7	0.39	20.34	17.34	0.34	0.0049371	0.34	393.44
B1078	24.35	1	-2.33	5.01	4.37	0.64	19.68	14.99		0.0062651	0.43	

B1090	19.21	0	-2.93	5.5	5.01	0.49	23.73	19.68		0.0063829	0.54	
B1099	18.52	0	-2.33	5.74	5.04	0.7	25.87	19.98	0.35	0.0088855	0.48	372.04
B1128	14.87	0	-0.92	5	4.26	0.74	19.63	14.23		0.0082954	0.34	
B113	20.8	0	-0.51	4.99	4.56	0.43	19.56	16.33	0.37	0.0035063	0.38	378.73
B1180	29.62	2	-1.36	5.12	4.73	0.39	20.55	17.56		0.0038516	0.41	
B1211	15.87	0	-2.61	5.41	4.92	0.49	22.99	19	0.28	0.0086746	0.48	403.02
B1257	21.1	0	-1.94	5.69	4.94	0.75	25.39	19.16	0.34	0.0085449	0.31	385.84
B1259	29.1	2	-1.28	6.09	5.21	0.88	29.13	21.35	0.37	0.0072475	0.46	403.5
B1389	16.47	0	-2.27	5.83	5.24	0.59	26.73	21.57	0.36	0.0035019	0.36	343.37
B1421	22.11	0	-1.23	4.85	4.12	0.72	18.45	13.35	0.41	0.008638	0.51	332.55
B1544	19.84	0	-1.94	4.45	3.99	0.46	15.54	12.51	0.34	0.0064452	0.4	369.32
B159	23.16	1	-1.36	5.21	4.43	0.78	21.32	15.41	0.34	0.0068682	0.46	410.29
B160	24.96	2	-2.6	5.47	4.79	0.68	23.49	17.99	0.35	0.0084541	0.4	381.39
B1618	31.08	2	-2.55	5.89	5.17	0.72	27.25	20.97	0.35	0.0058565	0.43	384.72
B1651	25.04	1	-2.61	5.21	4.76	0.45	21.31	17.82		0.0056083	0.32	
B1680	18.96	0	-2.82	5.27	4.79	0.48	21.83	18.02		0.0056949	0.47	
B1688	18.97	0	-1.71	4.61	4.08	0.53	16.69	13.08		0.0074928	0.43	
B1694	15.5	0	-2.37	5.67	5.01	0.66	25.22	19.71		0.0061711	0.49	
B1696	16.14	0	-2.34	5.35	4.88	0.47	22.49	18.68		0.0057091	0.36	
B173	16.88	0	-0.04	5.4	4.71	0.68	22.86	17.45		0.0067317	0.41	
B197	19.97	0	-0.57	6.18	5.66	0.53	30.02	25.13		0.0039238	0.55	
B221	24.08	1	-1.69	5.51	4.83	0.68	23.82	18.33		0.0055233	0.43	
B240	25.04	1	-0.88	5.97	5.31	0.66	27.98	22.15		0.0039983	0.38	
B288	16.21	0	-3.19	5.21	4.74	0.47	21.34	17.64		0.0062486	0.38	
B344	19.46	0	-2.84	5.13	4.41	0.72	20.68	15.29	0.31	0.0048651	0.43	419.6
B568	15.92	0	-1.75	5.42	4.74	0.68	23.07	17.66	0.39	0.0098115	0.38	386.21
B576	16.85	0	-2.7	5.49	4.9	0.59	23.7	18.86		0.0075383	0.4	
B579	21.28	1	-1.89	6.6	6.15	0.45	34.19	29.7		0.005174	0.5	
B645	32	2	-0.48	6.05	5.19	0.86	28.73	21.15	0.33	0.0062021	0.41	454.2
B669	16.65	0	-1.62	5.55	4.94	0.61	24.17	19.15		0.0073022	0.41	
B69	21.02	1	-0.86	5.43	4.86	0.57	23.12	18.54	0.33	0.0058226	0.39	421.76
B728	25.08	2	-2.79	5.56	4.96	0.6	24.25	19.3		0.0123026	0.46	
B878	20.68	1	-4.19	5.54	4.9	0.64	24.11	18.83		0.0058562	0.41	
B899	36.12	2	-1.39	6.73	5.95	0.78	35.59	27.79		0.0063138	0.46	

B904	18.66	0	-1.62	5.76	5.09	0.67	26.05	20.36		0.0102448	0.5	
B910	19.66	0	-0.82	5.59	4.98	0.62	24.56	19.44		0.0067699	0.4	
B921	19.33	0	-1.39	6.33	5.74	0.59	31.5	25.88		0.0051581	0.36	
B937	20.04	0	-1.08	5.73	5.06	0.67	25.8	20.08		0.0052002	0.38	
B944	24.93	1	-1.92	5.48	4.9	0.58	23.55	18.84	0.33	0.0037309	0.37	420.67
B957	19.32	0	-1.01	5.75	5.24	0.51	25.97	21.54		0.0032984	0.36	
B980	17.11	0	-1.87	5.71	5.15	0.55	25.56	20.83		0.0051689	0.4	
B992	20.91	0	-1.81	5.94	5.36	0.58	27.73	22.57		0.0049572	0.41	
B994	20.39	1	-3.28	5.54	4.89	0.64	24.06	18.81		0.0074793	0.36	
C115	28.52	2	-0.34	6.24	5.6	0.64	30.55	24.62	0.34	0.0043289	0.39	430.14
C139	22	1	-0.59	5.94	5.18	0.76	27.69	21.05		0.005076	0.38	
C22	18.53	0	-0.94	5.55	4.78	0.77	24.23	17.96		0.0092881	0.33	
C7	16.51	0	-0.92	5.22	4.48	0.73	21.36	15.79		0.0064682	0.47	
D108	20.37	0	-2.23	5.8	5.22	0.57	26.41	21.43	0.36	0.0061437	0.47	361.88
D1116	29.92	2	-1.86	5.54	4.8	0.75	24.14	18.08	0.32	0.0085335	0.48	391.7
D266	16.52	0	-0.22	5.34	4.66	0.68	22.38	17.02	0.38	0.0076866	0.42	367.82
D300	24.46	1	-0.85	5.33	4.69	0.65	22.35	17.24	0.35	0.0076481	0.4	408.81
D534	41.92	2	-0.37	6.1	5.44	0.66	29.19	23.2	0.34	0.0047674	0.51	448.34
D686	20.74	0	-2.13	6.1	5.63	0.46	29.18	24.92	0.32	0.0059859	0.4	402.85
D704	19.56	0	-1.93	5.77	5.17	0.59	26.1	21.03	0.37	0.0047344	0.41	346.59
E1137	20.31	0	-1.97	5.43	4.93	0.5	23.16	19.13	0.31	0.0045534	0.51	415.86
E199	22.42	1	-1.57	5.51	4.87	0.64	23.84	18.63	0.32	0.0048729	0.39	417.64
E231	20.15	0	-1.46	5.72	5.22	0.5	25.66	21.37	0.33	0.0050107	0.47	389.92
E236	37.17	2	-2.82	5.74	5.13	0.61	25.85	20.64	0.29	0.0045058	0.44	494.77
E307	21.24	0	-2.93	5.57	4.99	0.59	24.4	19.54	0.31	0.0047088	0.5	381.35
E315	22.1	1	-2.77	5.51	4.8	0.71	23.85	18.09	0.32	0.0060081	0.45	384.56
E396	21.04	0	-0.5	6.01	5.51	0.5	28.34	23.85	0.32	0.0028979	0.43	466.2
E442	16.47	0	-2.01	5.52	5.06	0.46	23.89	20.09	0.33	0.004337	0.39	417.61
E443	14.01	0	-1.43	5.83	5.31	0.51	26.65	22.18	0.33	0.0054029	0.41	373.82
E544	16.36	0	-0.01	5.18	4.72	0.46	21.07	17.52	0.37	0.0063872	0.33	362.66
E635	31.08	2	-3.18	5.4	4.81	0.6	22.9	18.18	0.27	0.0092814	0.37	480.47
E639	17.2	0	-3.18	5.18	4.73	0.45	21.1	17.58	0.31	0.0060393	0.45	363.28
E708	17.8	0	-0.74	5.09	4.49	0.6	20.36	15.81	0.34	0.0068063	0.42	411.44
E828	31.33	2	-2.76	5.62	5.02	0.6	24.77	19.8	0.37	0.00324	0.41	393.5

E871	18.61	0	-0.98	5.46	4.85	0.61	23.42	18.47	0.36	0.0045483	0.38	373.6
E876	19.35	0	-1.22	4.97	4.43	0.54	19.37	15.4	0.32	0.0048135	0.4	404.29
E927	17.32	0	-2.03	5.23	4.63	0.6	21.47	16.86	0.34	0.0054581	0.41	364.71